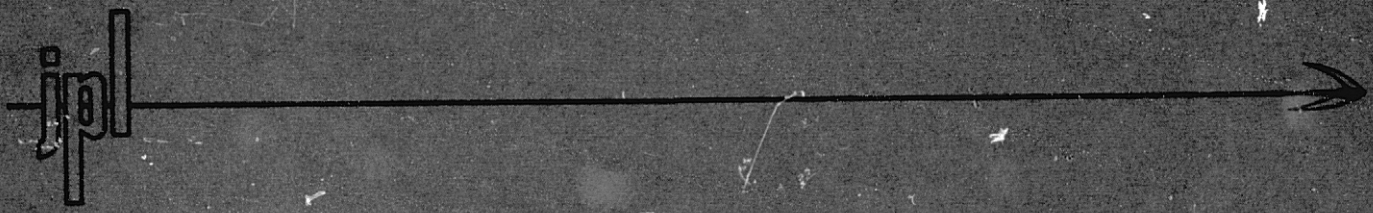

JET PROPULSION LABORATORY
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SECTION I

PLANETARY QUARANTINE FOR ADVANCED MISSIONS (NASA NO. 193-58-61-01)

Contents

Title and Related Personnel

Cognizance: C. Gonzalez, A. Hoffman

SECTION I

PLANETARY QUARANTINE FOR ADVANCED MISSIONS

1.1 INTRODUCTION

The overall objective of this task is to identify those areas of future missions which will be impacted by planetary quarantine (PQ) constraints. The specific objectives for this reporting period were 1) to perform an analysis of the effects of PQ on an outer planet atmospheric probe, 2) to prepare a quantitative illustration (i.e., a scenario) of spacecraft microbial reduction resulting from exposure to space environments. The Jupiter Orbiter Probe (JOP) mission was used as a model for both of these efforts.

1.2 SIGNIFICANT ACCOMPLISHMENTS

A PQ analysis was performed using a JOP mission as a model and an adaptation of the standard analysis techniques used in the past for the Mariner and the Viking Lander Missions. A probabilistic approach was used in performing the analyses. Current probabilities associated with microbial survival for outer planet missions were used as were current quarantine constraints and the probability of growth. The mission used as a model would have a launch in the 1981-1982 time frame. A detailed description of the effort is given in Appendix A. The conclusions drawn from the analysis indicate that standard spacecraft cleanliness and contamination control techniques will suffice to satisfy quarantine constraints except in the case of some selected materials and components.

For the microbial reduction scenario, the approach was to use the research data from the JPL Planetary Quarantine Project's Natural Space Environments Task and apply the data to a Jupiter Orbiter Probe Mission. The environments evaluated included the space vacuum, temperature (i.e., representative temperatures in and on the spacecraft), electromagnetic radiation, solar wind, galactic cosmic rays, solar particle events, and trapped radiation belts. A graphical representation of the definitions of where microbial burden can be found on spacecraft is shown in Fig. 1-1. The

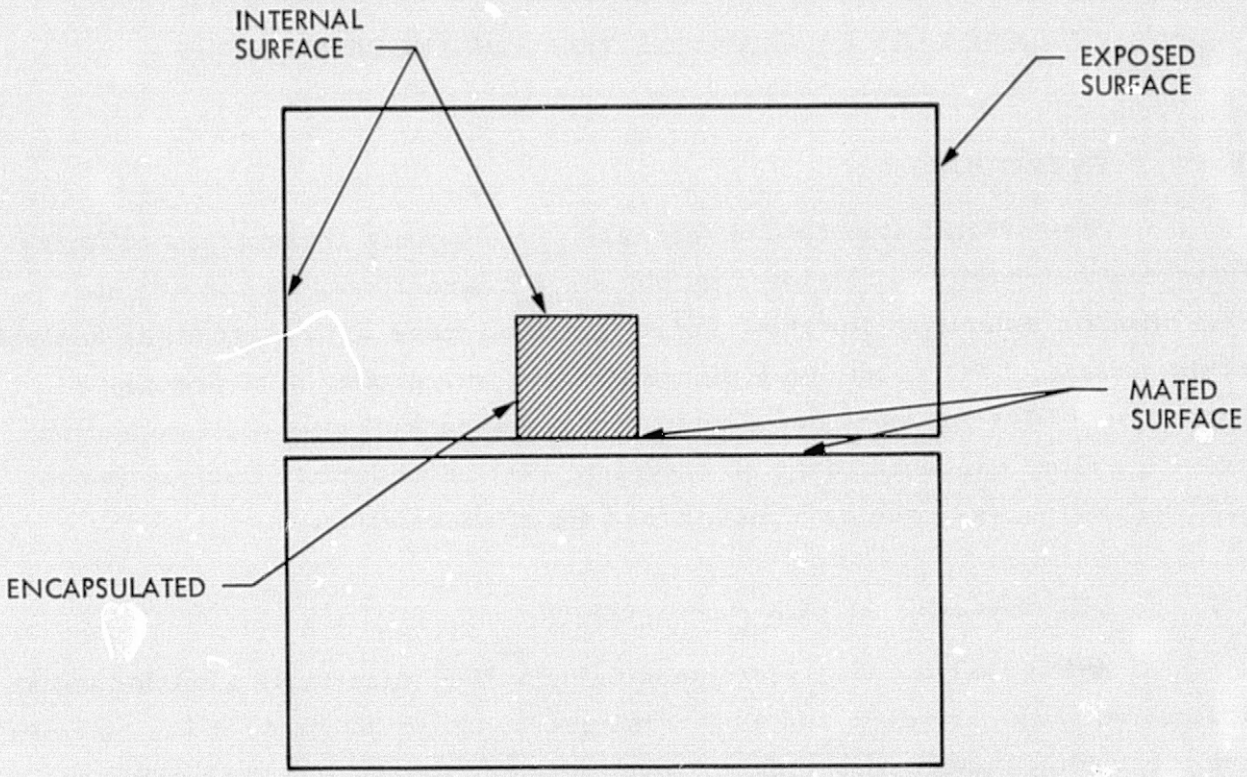

Fig. 1-1. Definitions of microbial burden on spacecraft

four types of burden are exposed, mated, internal surface, and encapsulated. Microbial reduction factors for launch and interplanetary environments are given in Table 1-1 and for Jupiter Trapped Radiation, in Table 1-2. Microbial burden as a function of mission phase is shown in Table 1-3. The launch burden estimates are based on Viking, Mariner and Pioneer experience.

From the scenario evaluation the following conclusions were drawn:

1) The Jupiter trapped radiation dominates microbial reduction,

2) Solar UV is significant on sunlit exposed surfaces,

3) Mated and internal surfaces have some reduction as a result of considering all environments, and

4) Encapsulated burden experiences no significant reduction.

Table 1-1. Microbial Reduction Factors, Expressed as Survival Fractions

Burden Type	Launch and Interplanetary Environments			
	Launch Pressure Decay	Temperature-Vacuum	Electromagnetic Radiation	Solar Wind
Exposed				
Sunlit	1.0	1.0	**0.01**	0.1
Shade	1.0	1.0	1.0	**0.1**
Mated	1.0	1.0	1.0	1.0
Internal Surface	1.0	1.0	1.0	1.0
Encapsulated	1.0	1.0	1.0	1.0

**Dominates lethality; number used in scenario

Table 1-2. Microbial Reduction Factors for Jupiter Trapped Radiation

Burden Type	Jupiter Trapped Radiation	
	Electrons	Protons
Exposed	**0.1***	0.1
Mated	**0.2**	1.0
Internal Surface	**0.2**	1.0
Encapsulated	1.0	1.0

*Orbiter, assume probe 10^{-3}

**Dominates lethality; number used in scenario

1.3 FUTURE ACTIVITIES

A level-of-effort planetary quarantine analysis will be performed on a representative advanced mission not previously studied. The approach will be to apply decision analysis methodology and compare the effectiveness of the approach with the conventional probabilistic approach.

Table 1-3. Microbial Burden as a Function of Mission Phase

Burden Location	Launch	Interplanetary		Planetary		
		Survival Fraction	Burden at Probe Release	Survival Fraction	Burden at Probe Entry	End 1st Orbit
Orbiter						
• Exposed						
• Sunlit	5×10^6	0.01	5×10^4	0.01	—	5×10^2
• Shaded	5×10^6	0.1	5×10^5	0.01	—	5×10^3
• Mated	1×10^6	1.0	1×10^6	0.2	—	2×10^5
• Interior	1×10^6	1.0	1×10^6	0.2	—	2×10^5
• Encapsulated	1×10^6	1.0	1×10^6	1.0	—	1×10^6
Probe						
• Exposed	1×10^6	0.1	1×10^5	0.001	1×10^2	—
• Mated	1×10^5	1.0	1×10^5	0.2	2×10^4	—
• Interior	1×10^5	1.0	1×10^5	0.2	2×10^4	—
• Encapsulated	1×10^5	1.0	1×10^5	1.0	1×10^5	—

SECTION II

NATURAL SPACE ENVIRONMENT STUDIES
(NASA NO. 193-58-61-02)

Contents	Title and Related Personnel
Subtask A Para. 2.1	EFFECT OF PLANETARY TRAPPED RADIATION BELT ON MICROORGANISM Cognizance: J. Barengoltz Associated Personnel: C. Myers
Subtask B Para. 2.2	EFFECT OF SOLAR WIND RADIATION ON MICROORGANISMS Cognizance: J. Barengoltz Associated Personnel: J. Brady (Bionetics) A. Ferreira (Bionetics)
Subtask C Para. 2.3	EFFECT OF SPACE VACUUM ON MICROORGANISMS Cognizance: M. Wardle J. Brady (Bionetics)
Subtask D Para. 2.4	PROBABILITY OF GROWTH IN PLANETARY ATMOSPHERES AND SATELLITES Cognizance: D. Taylor N. Divine Associated Personnel: A. Ingersoll
Subtask E Para. 2.5	EFFECT OF SOLAR ELECTROMAGNETIC RADIATION ON MICROORGANISMS Cognizance: M. Wardle Associated Personnel: D. Ross J. Brady (Bionetics)

SECTION II

NATURAL SPACE ENVIRONMENT STUDIES

2.1 EFFECT OF PLANETARY TRAPPED RADIATION BELT ON MICROORGANISMS

2.1.1 Subtask A Introduction

The objective of this subtask is to determine the effect of planetary trapped radiation belts on the survival of microorganisms associated with an unsterile spacecraft.

With fly-by missions now planned for Jupiter and Saturn and possible Jupiter orbiters and probes, the trapped radiation belts represent an environment lethal to microorganisms, and would reduce a requirement for decontamination of spacecraft before launch.

The major components of planetary trapped radiation belts are electrons and protons. The approach of the present task is to evaluate possible biological effects of these belts by subjecting spacecraft microbial isolates to different energies, exposures, and dose rates of those particles.

2.1.2 Significant Accomplishments

A contract for a study to determine the effect of electron radiation on encapsulated microorganisms was awarded and the work begun during this reporting period. This study will provide data to be compared with data for microbes on a surface, obtained previously under this task. The effect of factors not modeled in conventional radiation transport calculations will be investigated.

The effort to model the high energy electron data for cultured microbes previously obtained in this subtask (Refs. 1 and 2) has been completed during this reporting period. Several dose-dependent models of radiation sensitivity, both from the literature and developed under the subtask, have been investigated. The data fitting for these models was accomplished by a generalized computer program written as a part of the subtask. A complete report on the results of this activity may be found in Appendix B.

2.1.3 Future Activities

An analysis of the effect of secondary radiation will be performed with the new models, the encapsulated microbe data, and the electron transport program (previously modified and adapted for use on the JPL Univac 1108 computer system).

Planning will continue for the procurement of a study with high energy protons similar to those present in planetary trapped radiation belts.

2.1.4 References

1. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January – 30 June 1973, JPL Doc. 990-636, pp. 2-1 to 2-9, 1973.

2. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July – 31 December 1973, JPL Doc. 900-655, pp. 2-1 to 2-12, 1974.

2.2 EFFECT OF SOLAR WIND RADIATION ON MICROORGANISMS

2.2.1 Subtask B Introduction

The objective of this subtask is to determine the effect of solar wind radiation on microorganisms associated with nonsterile spacecraft.

This study is directed towards determining the radiation in spacecraft-associated microbial burden attributable to solar wind radiation. The data obtained will be utilized to update probability constants in the assessment of mission planetary quarantine constraints.

2.2.2 Approach

In order to fulfill the objectives of this task, an initial test program has been established to investigate the effect of solar wind electrons on test microorganisms held in a vacuum. A literature survey indicates biological effectiveness for electrons with energy in excess of 1 keV. Measurements and models of the solar wind electron spectrum imply an upper limit to the energy

range of interest at about 5 keV. Parametric tests in this energy range, 1-5 keV, will be conducted at accelerated dose rates to permit typical mission doses (fluences) in acceptable test durations. At each energy, tests with varying doses will be performed to obtain survival curves.

The first formal experimental phase consisted of tests with MM'71 isolates (sporeformers and non-sporeformers) and Staphylococcus epidermidis (SE) and spores of Bacillus subtilis var. niger (BSN) as comparative organisms. A second phase will be an analogous program with naturally occurring microbial populations as samples.

Additional tests with cultured organisms will be conducted:

1) To validate the accelerated tests, i.e., rate effect tests; and

2) To investigate the superposition principle for the total energy spectrum, i.e., combined energy (sequential) tests.

Finally a study of the trend, observed in the formal program on cultured microbes, for the survival curve to plateau at large fluences will be undertaken. Tests with more dilute inoculations and at higher electron energies will be conducted to investigate the possible role of sample clumping and non-biological contaminant shielding in this apparent saturation of lethality.

2.2.3 Significant Accomplishments

During this report period the following types of tests have been conducted on cultured microbes with electrons: rate effect tests, combined energy tests, and dilute inoculation sample tests. Naturally occurring microbes have been also studied by direct tests with fallout plates and with washed and filtered naturally occurring microbes.

A test program with cultured microbes at higher electron energies (10 and 20 keV) at another source at JPL has been initiated.

2.2.3.1 Rate Effect Tests. The rate effect tests were conducted on the Solar Wind Electron Source (SWES) (Ref. 1) and duplicated the microbiological procedures for the previous cultured organism tests (Ref. 2) and the 2 keV test conditions (Ref. 3) except for the electron flux. To search for rate effects,

the 2 keV tests on cultured microbes were repeated at a flux of 5×10^8 $cm^{-2}s^{-1}$, a factor of 100 lower than the previous tests. A summary of the results with previous data at the higher flux displayed is shown in Table 2-B. 1.

Table 2-B. 1. Flux (Rate) Experiment Results, 2 keV Survival Fractions

Flux =	Fluence = 2×10^{12} cm^{-2}		Fluence = 1×10^{13} cm^{-2}	
	5×10^8 $cm^{-2}s^{-1}$	5×10^{10} $cm^{-1}s^{-1}$	5×10^8 $cm^{-2}s^{-1}$	5×10^{10} $cm^{-2}s^{-1}$
Staphylococcus Epidermidis	0.04 ± 0.02	0.66 ± 0.87	0.04 ± 0.01	0.25 ± 0.47
Nonsporeformer Mean	0.54 ± 0.17	0.68 ± 1.2	0.21 ± 0.06	0.33 ± 0.62
Bacillus Subtilis Var. Niger	0.17 ± 0.04	0.23 ± 0.28	0.16 ± 0.10	0.03 ± 0.04
Sporeformer Mean	0.25 ± 0.04	0.30 ± 0.86	0.17 ± 0.01	0.075 ± 0.23

Due in part to the poor statistics, especially for the spacecraft isolate means which include a wide variety of radiation resistance, no statistically significant rate effect was found.

2.2.3.2 Combined Energy Tests. In order to apply the type of data obtained at discrete energy levels to a prediction of lethality for the environment with an energy spectrum, some rule for the accumulation of effect must be assumed. For the simple model giving the logarithm of the survival fraction as a linear function of the fluence at one energy, the superposition principle would yield the linear superposition of a series of discrete functions for a multiple discrete energy exposure. The superposition principle would also predict the spectrum case; here the linear superposition would be an integral over energy rather than a sum.

The purpose of these tests was to examine the validity of the superposition principle for electrons of varying energy (2, 3, and 4. 5 keV). Accordingly, multiple discrete energy exposures were conducted during this report period on the microbes used in the program. The microbiological procedures were unchanged from Ref. 2. The three test conditions, each run conducted twice, are shown in Table 2-B. 2. The results are summarized in Table 2-B. 3.

Table 2-B.2. Matrix for Total Spectrum Simulation

Run	Energy, KeV	Flux, cm^{-2} sec^{-1}	Duration, sec	Fluence, cm^{-2}
A	2.0	5×10^{10}	60	3×10^{12}
	3.0	1×10^{10}	40	4×10^{11}
	4.5	5×10^{9}	20	1×10^{11}
				Total 3.50×10^{12}
B	2.0	5×10^{10}	300	1.5×10^{13}
	3.0	1×10^{10}	200	2.0×10^{12}
	4.5	5×10^{9}	100	5.0×10^{11}
				Total 1.75×10^{13}
C	2.0	5×10^{10}	1500	7.5×10^{13}
	3.0	1×10^{10}	1000	1.0×10^{13}
	4.5	5×10^{9}	500	2.5×10^{12}
				Total 8.75×10^{13}

Table 2-B.3. Combined Energy Results, Survival Fractions

Organism	Run A*	Run B	Run C
Staphylococcus Epidermidis	0.009	0.003	0.001
Nonsporeformer Mean	0.23	0.06	0.02
Bacillus Subtilis Var. Niger	0.16	0.06	0.015
Sporeformer Mean	0.31	0.26	0.05

*See Table 2-B.2 for run physical parameters

A comparison of these results with the predictions based on one energy level (Ref. 3) indicates that the superposition principle does not apply to the organisms and physical environments of this program. In general the superposition prediction underestimates the survival fraction (overestimates lethality).

2.2.3.3 Dilute Inoculation Tests. In order to investigate the possible effect of organism clumping in the observed saturation of lethality at the higher fluences, some of the tests of the formal cultured microbe study were repeated

during this report period with inoculations of 10^4 microbes/cm^3 instead of the standard, 10^6. If clumping is an important factor, the survival fractions would be expected to be smaller for these dilute inoculations as clumping would be reduced.

The results of these tests are summarized in Table 2-B. 4. If any effect at all was observed, the survival fractions were larger for the dilute inoculation tests. Unfortunately the statistics are too poor to allow any statistically valid conclusions to be drawn.

Table 2-B. 4. Dilute Inoculation Experiment Results, Survival Fractions

Energy, keV	3.0		4.5	
Fluence, cm^{-2}	5×10^{12}	1×10^{13}	2×10^{12}	5×10^{12}
Staphylococcus Epidermidis				
Dilute	0	0	0	0
Standard	0	0	0	0
Nonsporeformer Mean				
Dilute	0.14 ± 0.33	0.15 ± 0.28	0.062 ± 0.12	0.051 ± 0.11
Standard	0.062 ± 0.12	0.059 ± 0.10	0.042 ± 0.097	0.023 ± 0.052
Bacillus Subtilis Var. Niger				
Dilute	0.044 ± 0.072	0.034 ± 0.049	0.024 ± 0.038	0.022 ± 0.043
Standard	0.0041 ± 0.0061	0.0064 ± 0.0080	0.0038 ± 0.0047	0.0068 ± 0.010
Sporeformer Mean				
Dilute	0.015 ± 0.043	0.020 ± 0.064	0.0097 ± 0.027	0.014 ± 0.041
Standard	0.018 ± 0.060	0.0093 ± 0.026	0.0092 ± 0.026	0.010 ± 0.032

2.2.3.4 Naturally Occurring Organism Tests

During this reporting period two types of tests with naturally occurring microbes were conducted. In both cases fallout samples were first collected on twelve 4" x 4" stainless steel plates (pre-sterilized) lying in a large sterile stainless steel tray. The collection tray was placed on top of a tall cabinet in the center of a cable harness assembly area and exposed for approximately six days.

For the untreated natural fallout tests, the twelve plates were randomly segregated into three groups of four plates: exposed, dark control, and bench control. The three exposed plates were mounted on the exposed side of the SWES test fixture (Ref. 1), and the three dark control plates on the opposite side. This fixture had been modified to allow this procedure. The bench controls

were held in the laboratory and assayed with the other plates. The post-exposure recovery and assay procedure was as in previous tests (Ref. 2). The test results are summarized in Table 2-B.5.

Table 2-B.5. Survival Fractions for Untreated Natural Fallout

Energy, keV	1.5		4.5		
Fluence, cm^{-2}	5×10^{14}	1×10^{15}	2×10^{12}	5×10^{12}	2×10^{13}
Relative to Dark	0.70	0.67	0.60	0.43	0.46
Relative to Bench	0.32	0.74	0.24	0.21	0.31

The results indicate that for these highly contaminated plates, the lethality was quite small. Note that these fluences were larger than those employed in the test program for cultured microbes (Ref. 3). These results are probably due to the unrealistically large amount of non-biological contaminants (relative to a spacecraft) found on these plates by microscopic examination, which shield the microbes.

Therefore another series of experiments was conducted with washed and filtered natural fallout. In this experimental design, all twelve fallout plates were sonicated in a common bath. Then the sonicate was filtered, centrifuged and finally resuspended in sterile distilled water. Ten exposed, ten dark control and ten bench control sample tacks were then inoculated. At this point the experiment was completed as in the case of the cultured microbe tests (Ref. 2).

Tests have been completed at 2, 3, and 4.5 keV. The results are displayed in Figs. 2-B.1, 2-B.2, and 2-B.3. For comparison some of the data from the cultured microbe tests are also shown. It is clear from the 2 and 3 keV data that washed and filtered natural fallout behaves like spacecraft isolate No. 5. The results at 4.5 keV indicate some sort of error and cannot be explained. The tests will undoubtedly have to be repeated.

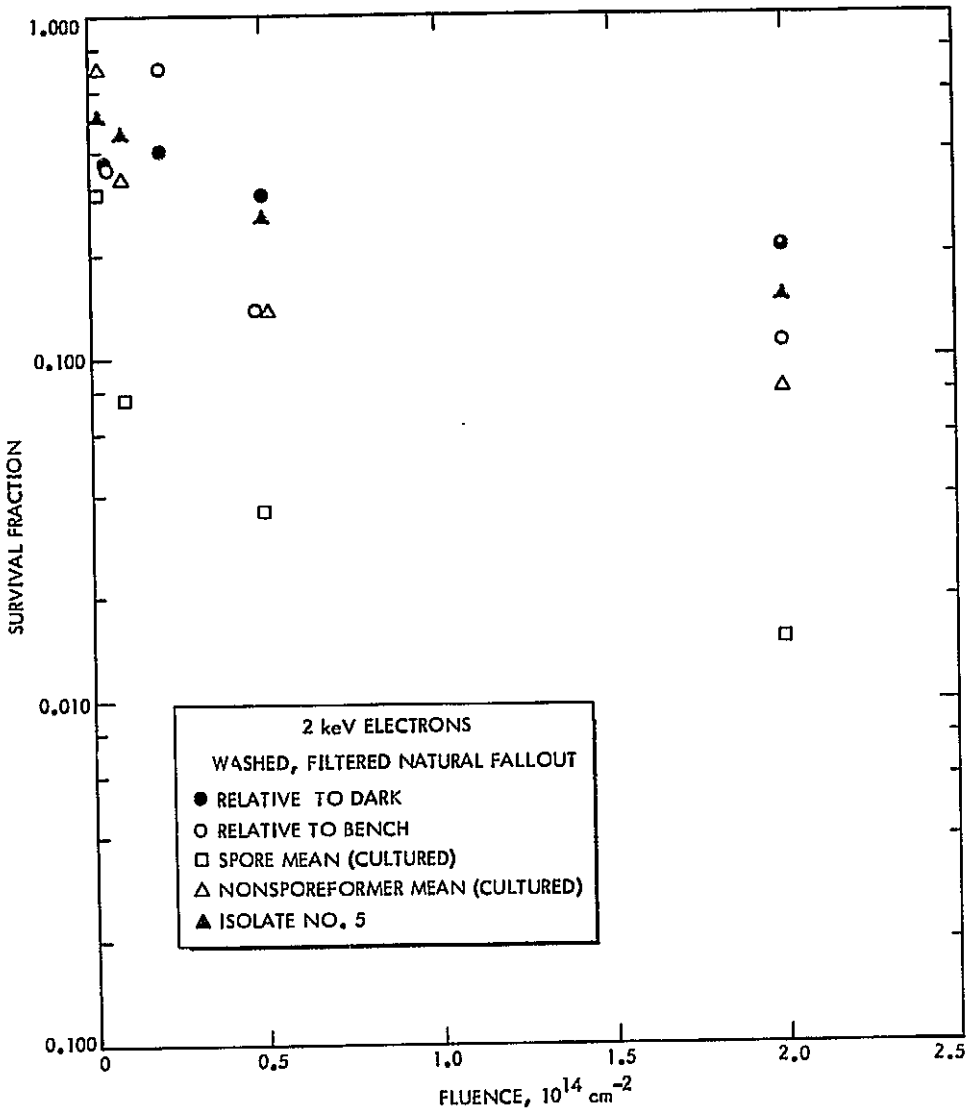

Fig. 2-B.1. Survival fractions for washed and filtered naturally occurring microbes exposed to 2 keV electrons

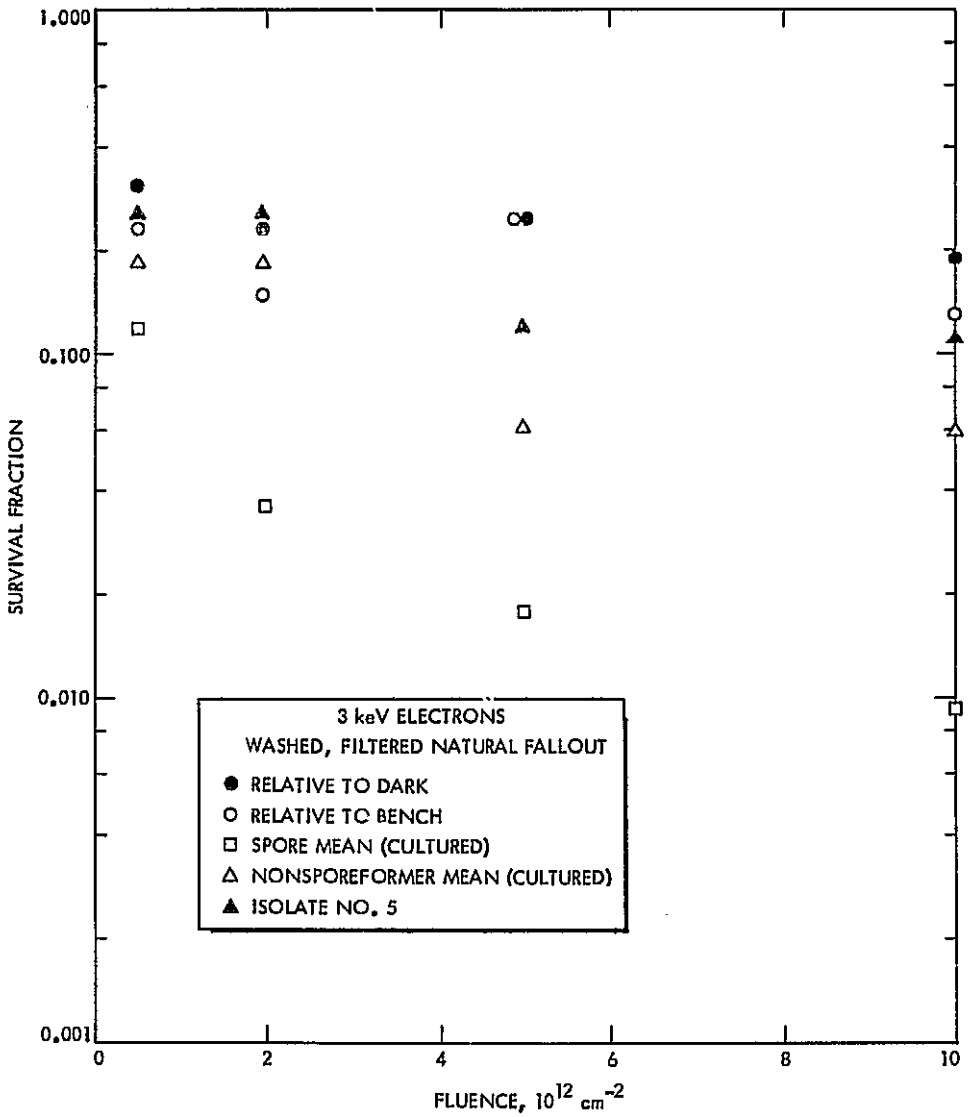

Fig. 2-B.2. Survival fractions for washed and filtered naturally occurring microbes exposed to 3 keV electrons

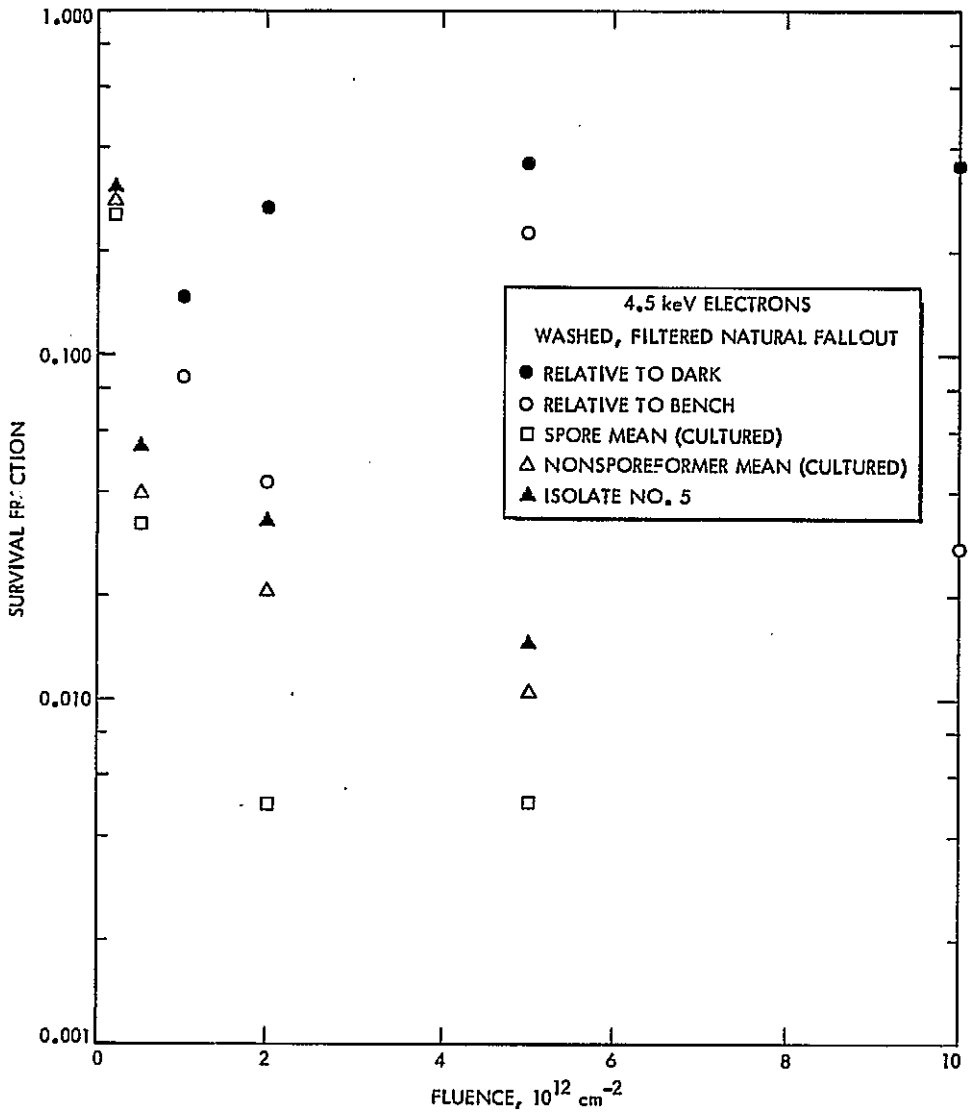

Fig. 2-B.3. Survival fractions for washed and filtered naturally occurring microbes exposed to 4.5 keV electrons

2.2.4 Future Activities

The data from the cultured microbe tests will be modeled.

The tests at 10 and 20 keV, already initiated, will be completed.

New microbiological procedures to obtain consistent samples of naturally occurring organisms will be sought. If successful the formal test program with electrons will be conducted.

2.2.5 References

1. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July – 31 December 1974, JPL Doc. No. 900-701, 1975.

2. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January – 30 June 1975, JPL Doc. 900-715, 1975.

3. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July – 30 December 1975, JPL Doc. No. 900-732, 1976.

2.3 EFFECT OF SPACE VACUUM ON MICROORGANISMS

2.3.1 Subtask C Introduction

The objective of this task is to determine the effect of extended exposure to space vacuum and spacecraft temperatures on the viability of microbial species representative of those found on spacecraft.

Earlier work in this task area concentrated on the study of bacterial isolates from MM'71 spacecraft (JPL Documents 900-597, 1972; 900-608, 1973; and 900-675, 1974). These isolates, along with Bacillus subtilis var. niger and Staphylococcus epidermidis (ATCC 17917), were exposed to a vacuum of 10^{-7} torr at temperatures of -40 to 75 °C for durations from 7 to 187 days. In general, it was found that a significant vacuum/temperature effect occurred at temperatures of 40 °C and greater for all durations studied. In addition, spores evidenced greater survival than did nonsporeformers.

Previous studies of the environmental microbiology of spacecraft assembly areas (e.g., Taylor, et. al. 1970, Dev. Indust. Micro. 11: 225-240), have indicated that approximately 90% of the naturally occurring microbes in such areas are nonsporeformers. Although technical difficulties exist in the investigation of the response of naturally occurring microbes to environmental stress, the significance of these populations is greater in terms of quarantine considerations. It was therefore decided that the study of the vacuum/temperature effect on naturally occurring microorganisms would make more complete the understanding of this parameter of the natural space environment.

2.3.2 Significant Accomplishments

An additional test of the resistance of pure cultured and naturally occurring organisms was conducted per the technique previously described (JPL Semi-Annual Review, Document 900-732, April 18, 1976). The results of all the comparative tests are shown in Table 2-C.1. In relating the data of test 3 with that previously reported (tests 1 and 2), a corroboration of the greater vacuum/temperature resistance of naturally occurring microbes as compared to a pure culture of Bacillus subtilis var. niger is evident.

Table 2-C.1. Survival Fractions of Organisms Exposed to Space Vacuum (10^{-6} torr, 75°C, 7 days)

Test	Pure Cultured*	Naturally Occurring
1	0.034 (±0.014)**	0.453 (±0.012)
2	0.008 (±0.007)	0.504 (±0.050)
3	0.008 (±0.007)	0.523 (±0.190)

*Bacillus subtilis var. niger
**Standard Deviation

Taxonomic characterization of naturally occurring isolates surviving the vacuum/temperature exposure was also conducted during this reporting period. A random selection of isolates was obtained. All colonies were sequentially numbered on an entire series (test acquired) of agar contact plates. A random numbers table was used to select a total of 60 isolates

from two 1/4 ft^2 naturally occurring microorganism test surfaces. The isolates selected in this manner were streaked on Trypticase Soy Agar (TSA) to obtain pure cultures and stored on TSA slants for additional testing. The isolates were separated into taxonomic groups using the identification schemes for microorganisms isolated from Apollo and unmanned spacecraft.

A summary of the taxonomic groups is shown as Table 2-C.2. Approximately 42% of the isolates surviving the vacuum temperature environment were gram positive cocci while about 7% were Bacillus species. The significant portion of gram negative rods (20%) and diptheroids (13%) observed tends to emphasize that a large human source natural population was collected. No statement can be made on any relative differences in resistance among the different species present in the sampling environment owing to the fact that a classification of control sample isolates was not conducted.

Table 2-C.2. Summary of Taxonomic Groups Surviving Vacuum/Temperature Exposure

Group	Number of Isolates	Percentage of Sample
Actinomycetes	5	8.33%
Bacillus Species	4	6.67
Diptheroid	8	13.33
Fungi	2	3.33
Gram Negative Rod	12	20.00
Micrococcus Group 7	5	8.33
Micrococcus Group 8	2	3.33
Staphylococcus Group 2	3	5.00
Staphylococcus Group 3	1	1.67
Staphylococcus Group 4	9	15.00
Staphylococcus Group 5	2	3.33
Staphylococcus Group 6	5	8.33
Yeast	2	3.33

2.3.3 Future Activities

No future activities are planned for this subtask.

2.4 PROBABILITY OF GROWTH IN PLANETARY ATMOSPHERES AND SATELLITES

2.4.1 Subtask D Introduction

The objectives of this subtask are to relate environmental parameters affecting microbial growth to conditions present in the atmospheres of Jupiter and Saturn, and to identify and study satellites of Jupiter and Saturn having possible biological interest.

2.4.2 Significant Accomplishments

A contract with the California Institute of Technology to continue the participation in this task of Dr. Andrew P. Ingersoll (Division of Geological and Planetary Sciences) has been made. Observational and theoretical descriptions of the structure and dynamics of the atmospheres of Saturn and Uranus will be applied to the derivation of the probability of growth.

A survey of all observed spot motions in Saturn's atmosphere, to determine wind speeds and mechanisms, and an estimate of the internal energy required to suppress meridional transport, have been completed. These results suggest that most qualitative aspects of Jupiter's atmosphere apply to Saturn as well, so that the application of the dynamic modeling developed for Jupiter will be valid for Saturn with suitable changes in parameter values.

2.4.3 Future Activities

The foregoing work will be repeated for Uranus, with the possible conclusion that its atmospheric circulation is rather different from Jupiter's.

For both planets, the vertical structure of the atmosphere (profiles of pressure, temperature, altitude, clouds, etc.) will be derived from theory and observations recently published. The dynamical theory of Jupiter's atmospheric circulation will be applied (using computer simulation) to Saturn to determine

vertical mixing rates, residence times, and probability of growth. A modified, possibly simplified and less certain, dynamical treatment will yield comparable results for Uranus as well.

2.5 EFFECT OF SOLAR ELECTROMAGNETIC RADIATION ON MICROORGANISMS

2.5.1 Subtask E Introduction

The objective of this task is to estimate the effect of solar electromagnetic radiation (SER) on the survival of microbial populations in a space environment. Efforts will be addressed to the investigation of the photobiological effect of SER in a fashion that permits direct transference of the results to considerations of planetary quarantine. Such information will enable the updating of probability constants in the assessment of applicable planetary quarantine constraints for a mission.

2.5.2 Approach

The approach for this task involves the subjection of test species to SER in a manner that will yield interpretive data on the response of spacecraft biocontaminants to the SER of space. Primarily this entails the high vacuum irradiation of microorganisms, pure cultured and naturally occurring, with broad spectrum SER (far ultraviolet through infrared). Pure cultured species are studied to define the effect of SER under different dose, dose rate and temperature conditions. Naturally occurring populations are collected and exposed to SER in a natural state; i.e., no laboratory treatment of the organisms is instituted prior to test environment exposure.

2.5.3 Significant Accomplishments

Our previous studies of the effect of solar electromagnetic radiation on microorganisms have provided evidence that a residual population of organisms survive irradiation as a consequence of their being shielded from lethal spectra (i.e., ultraviolet). Such shielding (depending on the experiment) could have been produced by viable or nonviable particulates as well as structural elements of the irradiation test fixture (e.g., solar cell electrical leads).

Therefore, experiments were performed in order to better understand the nature of this residual survivability.

As reported in the previous semi-annual review (JPL Document 900-732), the radiation resistance of cultured, naturally occurring survivors was not significantly different ($P < 0.05$) from that observed for MM'71 isolates (spores and nonsporeformers). Therefore, pure cultures of naturally occurring organisms were employed in an investigation of the effect of viable cell shielding on resistance. Test populations were prepared as previously described (JPL Document 900-732) and deposited on aluminum stages as dilute inocula, thus yielding a sparser distribution of cells on the irradiated surfaces. It was hypothesized that the dilute inocula would evidence less shielding due to cell clumping (stacking) and as a consequence exhibit a lesser resistance to irradiation. The results of these tests are shown as Table 2-E.1. From these data it can be concluded that, for the cited experimental conditions, diluting the inoculum does not predictably alter the survival fraction.

Table 2-E.1. Effect of Inoculum Concentration on Solar Electromagnetic Radiation Resistance

Solar Constant,[a] Temp (°C)	Spores		Nonsporeformers	
	Dilute	Nondilute	Dilute	Nondilute
0.1, -125 °C	3.8×10^{-2}[b] (9×10^{2})[c]	1.1×10^{-1} (1×10^{5})	1.6×10^{-1} (1×10^{3})	3.4×10^{-2} (3×10^{4})
0.5, -15 °C	3.1×10^{-1} (1×10^{3})	8.3×10^{-2} (1×10^{5})	2.5×10^{-1} (7×10^{2})	2.5×10^{-2} (2×10^{5})
1.0, +70 °C	3.5×10^{-2} (1×10^{3})	8.6×10^{-2} (5×10^{4})	8.5×10^{-2} (2×10^{2})	4.9×10^{-2} (7×10^{4})

[a] Dose = 2×10^{2} ergs mm^{-2} (200 - 270 nm)
[b] Survival Fraction
[c] Approximate inoculum concentration

A subsequent series of experiments was performed to study the controlled deposition of particulate contamination as a determinate of survivability. The basic procedure for these tests involved the inoculation of test and control aluminum stages with pure cultures of spores. Half the inoculated stages were exposed to environmental particle fallout for up to one week while the other half were held in the same environment, protected from particulate contamination. The results of these tests are shown as Table 2-E.2. As can be seen, the stages contaminated with spores and particles on an average evidenced a slightly higher survival fraction than did those having only spores on their surfaces; however this was not statistically significant ($P < 0.05$). Spores averaged approximately an 8.5% greater survivability in the presence of particles as compared to spores alone; discounting the 400 ergs mm^{-2} test (which appeared anomalous for the spore stages) this percentage dropped to 1.7. Survival fractions were also calculated for viable particles collected on stages. The resistivity of naturally occurring microbes associated with particles was seen to be much greater than for cultured spore populations (with or without particles) and much less sensitive to increasing doses. This fully reflects observations previously made in this task and points to the difficulty of trying to distinguish between shielding effects and innate resistance when investigating naturally occurring populations. Based on the data we have collected it does not appear that shielding due to the particles associated with naturally occurring microorganisms alone accounts for their observed greater resistance to solar electromagnetic radiation: innate resistance of these populations, greater than that of pure cultured cells, seems also to be a logical conclusion.

Table 2-E.2. Effect of Particulate Contamination on Solar Electromagnetic Radiation Resistance

Dose[a] (ergs mm^{-2})	Spores	Spores + Particles	Viable Particles
200	2.1×10^{-1}[b]	2.0×10^{-1}[c]	6.0×10^{-1}
400	3.8×10^{-3}	2.0×10^{-2}	5.6×10^{-1}
800	1.1×10^{-2}	2.3×10^{-2}	3.6×10^{-1}
1,000	1.1×10^{-2}	8.8×10^{-3}	—
2,000	1.3×10^{-3}	3.2×10^{-3}	2.5×10^{-1}

[a]Solar Constant = 1.0 sun
[b]Survival Fraction
[c]Corrected for viable particles

Table 2-E. 3 shows a characterization of the particulate contamination observed in these tests (Table 2-E. 2). Both filters and stages were employed to estimate the type and number of particulates falling out into the test surfaces (ASTM standard F50-69 was followed with slight modifications).

An attempt was made to amplify data acquired on the resistance of naturally occurring populations collected on solar cell surfaces. As discussed in previous reports on this task area, solar cells were selected as collection surfaces owing to their complex surfaces which, it was assumed, would be more conducive to shielding protection of microbes and hence a conservative estimate of their resistance (which could be related to flight hardware). Since this shielding factor was strictly an assumption, it was described worthwhile to run a series of comparative tests between solar cell fixtures and smooth, flat aluminum fixtures (protocol was per JPL Documents 900-701, 900-715). As can be seen in Table 2-E. 4, identical survival fractions were obtained for both types of collection surfaces thus indicating that under the conditions imposed no additional shielding due to the irregular solar cell surfaces was evident.

Additional efforts during this reporting period have been directed toward developing an identification protocol for naturally occurring bacterial isolates. A workable scheme has been developed in the form of a dichotomous key with corresponding cultural and biochemical procedures, integrated from several existing schema.

To assist in data reduction and interpretation of the numerous tests that must be performed for each isolate, a computer program has been written which has the capability of discriminating among several "best fit" choices, and presenting each candidate identification with its number of discrepant tests. By use of this program, a significant reduction in required manhours has been achieved in analyzing large numbers of isolate identification test data. A taxonomic characterization of randomly selected survivors of naturally occurring tests (Table 2-E. 4) is shown as Table 2-E. 5.

Table 2-E. 3. Natural Particulate Fallout Count for Solar Electromagnetic Tests

A. Controls

Stage	Count at Size (μ)	Description
1	0	
2	2 at 10	White Crystalline
	3 at 15	White Crystalline
	1 at 25	White Crystalline
	1 at 35	White Crystalline
	1 at 90	White Crystalline
3	3 at 25	White Crystalline
	1 at 45	White Crystalline

Filter

1	0	
2	1 at 175	White Crystalline
	1 at 110	White Crystalline
3	2 at 20	Clear Crystalline
	2 at 25	White and Black Crystalline
	2 at 50	Clear Crystalline
	2 at 15	Clear and Orange Crystalline
	2 at 5	Black or Metallic

B. Test

Size Range (μ)	Stage[a] 1	2	3
0 - 5	TNTC	TNTC	TNTC
5 - 25	2093	2671	1725
25 - 50	574	624	427
50 - 100	155	223	76
100 - 200	86	51	25
>200	41	13	23

Size Range (μ)	Filter[b] 1	2	3
5 - 25	2397	3269	2961
25 - 50	1082	987	1833
50 - 100	451	1128	491
100 - 200	93	156	705
>200	139	423	564

[a]Crystalline and fiber particulates
[b]Crystalline and fiber particulates with metallics noted on filter no. 3.

Table 2-E. 4. Effect of Surface Configuration on the Resistance of Naturally Occurring Microbial Fallout to Solar Electromagnetic Radiation*

Survival Fraction (Number of Tests)	
Solar Cell Fixture	Flat Aluminum Fixture
0. 04 (2)	0. 04 (3)
*Approximately 4 x 10^4 ergs mm^{-2} at 0. 5 sun, 70 °C	

Table 2-E. 5. Naturally Occurring Survivors of Exposure to Solar Electromagnetic Radiation*

Isolate No.	Identification
1	Bacillus megaterium
2	Bacillus cereus
3	Bacillus brevis
4	Bacillus pumilus
5	Bacillus megaterium
6	Gram negative rod
7	Diptheroid
8	Micrococcus (Group 7)
9	Micrococcus (Group 7)
10	Diptheroid
11	Staphylococcus (Group 4)
12	Bacillus polymyxa
13	Bacillus pumilus
14	Diptheroid
15	Diptheroid
16	Bacillus megaterium
*Approximately 4 x 10^4 ergs mm^{-2} at 0. 5 sun, 70 °C	

In summary, the data indicates that naturally occurring microorganism resistance to solar electromagnetic radiation can't be fully understood by the simulated attempts to control shielding that were employed. While future experiments employing naturally occurring (noncultured) microbes may be useful in elucidating their innate resistance it remains an even greater challenge to understand the natural association of such cells with environmental

particulates that provide protective shielding. Whatever the combinatorial factors of innate resistance and particulate shielding are in the resistance of natural populations to broad spectrum nonionizing radiation, it remains a known that these populations are less sensitive than their cultured counterparts, and hence they should be reckoned with in estimating the quarantine significance of this parameter of the natural space environment.

2.5.4 Future Activities

No future activities are planned in this task area.

SECTION III

QUARANTINE RISK ASSESSMENT METHODOLOGY (NASA NO. 193-58-61-06)

Contents	Title and Related Personnel
Para. 3.1	QUARANTINE RISK ASSESSMENT METHODOLOGY
	Cognizance: A. Hoffman, W. North (SRI)
	Associate Personnel: M. Merkhofer (SRI) D. Quinn (SRI)

SECTION III

QUARANTINE RISK ASSESSMENT METHODOLOGY

3.1 QUARANTINE RISK ASSESSMENT METHODOLOGY

3.1.1 Introduction

The overall objective of this task is the application of decision analysis methodology in the investigation of complex planetary missions in order to quantify the trade-offs between satisfying planetary quarantine (including Earth) constraints and mission objectives. The specific objective is the application of this methodology to a Mars Sample Return Mission (MSRM) in order 1) to develop a conceptual framework for evaluating such missions, 2) to perform a sensitivity analysis for a "reference" Mars sample return mission, and 3) to develop techniques for determining and assessing contamination penalties for sample return missions.

3.1.2 Significant Accomplishments

The approach being used consists of developing models to relate the outcome of certain events to basic decision variables, using probability theory to describe uncertain quantities. Then, a first order sensitivity analyses are performed for the purpose of reducing the number of branches of the decision "tree." Those branches remaining are subjected to a detailed numerical evaluation.

A conceptual framework has been designed to evaluate the MSRM in order to address both the likelihood and magnitude of possible back contamination (BC). A model has been developed to assess the probability of BC from various spacecraft sources containing Martian organisms. Contamination of the Earth is assumed to result from release of an organism into the Earth's environment. The model is based on work described in reference 1 and is expressed in the form of probability trees which identify the risk elements. The probability trees are used to analyze the effectiveness of strategies or devices to reduce risk due to these elements.

Example cases have been performed using a mission designed to return multiple unsterilized samples to Earth via a Mars orbit rendezvous with direct Earth entry. Several contamination modes are being considered including:

1) Release of organisms originating on the surface of the return vehicle (i.e., surface contaminants);

2) Leakage of organisms sealed within the sample canister or sample compartment;

3) Major equipment failure (e.g., non-nominal entry of the Earth entry capsule causing release of organisms).

The model gives the contamination paths of the organisms using assumed probabilities of occurrences of given events, such as leakage of the sample compartment. A probability tree is being developed for computing risk of contamination due to equipment failure. Each path through the tree represents a unique sequence of events resulting either in contamination or no contamination. Paths through the tree which lead to contamination are assumed to result in the exposure of the Earth's environment to the foreign bioload of the return vehicle. The model can also be used to evaluate the effectiveness of various fail safe mechanisms to reduce the probability of contamination. This is done by modifying the tree structure to account for the reliability of the fail safe mechanisms. Then trade-offs between imposing a fail safe strategy and losing valuable scientific information, such as would result from sample sterilization, can be evaluated.

3.1.3 Future Activities

The current contracted effort on MSRM will continue until February, 1977, when a report will be published.

3.1.4 Reference

Yen, C. L., "Back Contamination System Model," JPL Engineering Memorandum 393-233, November 1, 1974.

SECTION IV

POST LAUNCH RECONTAMINATION STUDIES (NASA NO. 193-58-62-03)

Contents

Title and Related Personnel

POST LAUNCH RECONTAMINATION STUDIES

Cognizance: J. Barengoltz

Associate Personnel: I. Ickovits (Bionetics)

SECTION IV

POST LAUNCH RECONTAMINATION STUDIES

4.1 POST LAUNCH RECONTAMINATION STUDIES

4.1.1 Introduction

The objective of the task is the development of an analytical technique for the evaluation of the probability of the relocation of particles from non-sterile to sterile areas on a spacecraft. The recontamination process is important for all multiple missions with separate microbiological burden allocations for various major spacecraft systems, and critical for life detection experiments that risk contamination from nonsterile components.

The current specific goal is the recontamination hazard for shuttle (STS) – launched planetary spacecraft. In this case the relocation of interest is transfer from the orbiter to the spacecraft prior to or immediately after separation.

The approach has been to study the effects of typical mission environments on the redistribution of particles on spacecraft surfaces both analytically and experimentally. This study consists of three logical components, which have been reflected in the effort: (1) particle adhesion, (2) dynamic release mechanisms, and (3) particle transport. The effort in particle adhesion has been principally a particle release experiment, together with analytical work and attempts to correlate other data found in the literature and elsewhere. Under dynamic release mechanisms, meteroid impact and pyro firing have been modeled The particle transport activity is an analytical effort which includes the development of codes for spacecraft geometry and orientation, forces acting on released particles, and trajectory. In addition to the three areas discussed above, a new component (4) is being considered, namely the creation of new particulates (as opposed to pre-existing surface contamination).

All of these components will finally be assembled into an operational, integrated computer code which provides estimates for a particular mission type.

4.1.2 Significant Accomplishments

4.1.2.1 Particle Adhesion. During this reporting period an experimental activity to augment the current particle adhesion model has been completed. Data has been obtained for a direct comparison of the adhesion at atmospheric pressure, partial vacuum (10 mm Hg) and high vacuum (10^{-5} mm Hg) of glass beads and dust to both glass and aluminum surfaces.

The first phase included five size ranges of glass beads analogous to previous work at partial vacuum (Ref. 1) at accelerations ranging from 100 gee to 30 kilogee.* The atmospheric pressure and high vacuum runs are performed simultaneously in a centrifuge. The evacuated centrifuge tubes are obtained through the use of a fixture shown in Figure 4-A.1 in conjunction with an oil diffusion pump vacuum system. A schematic of the sample in the test configuration in the centrifuge is presented in Figure 4-A.2. The particles are counted prior to and after testing by microphotography. A more detailed description of the procedures may be found in Ref. 2.

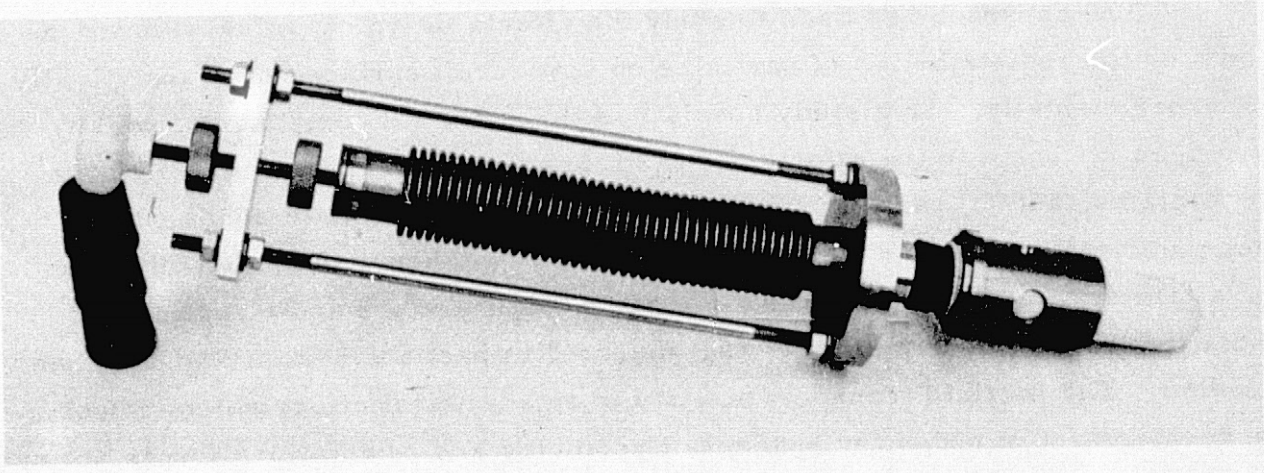

Fig. 4-A.1. Device to accomplish sealing of sample container through wall of vacuum chamber

*1 gee is the acceleration due to gravity at the surface of the Earth (9.8 ms^{-2}).

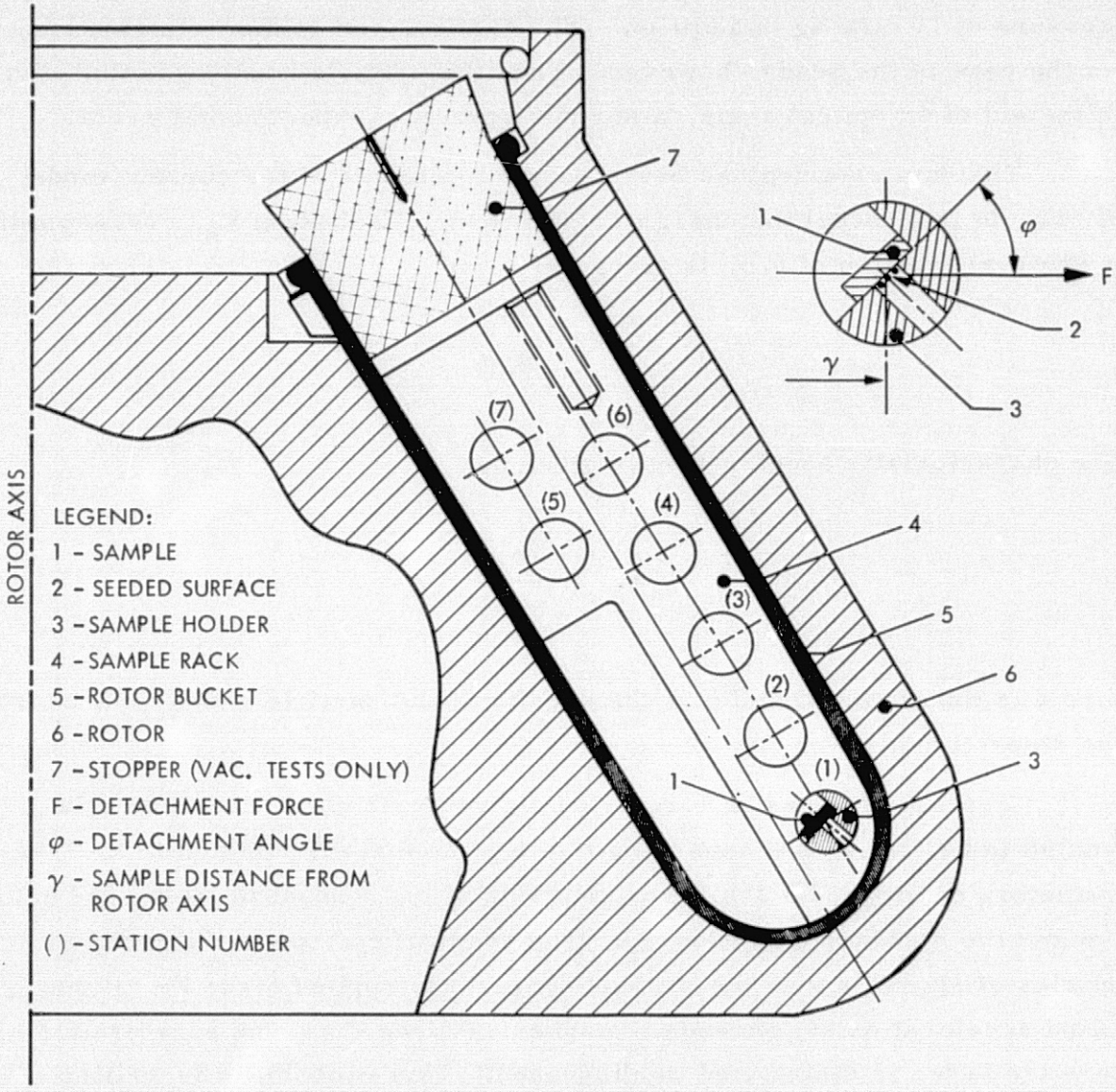

Fig. 4-A. 2. Sample installation and nomenclature

The second phase consisted of analogous tests with dust, with tests at a pressure of 10 mm Hg in addition. The dust was not in discrete size ranges as in the case of the beads, however. Thus the particle counting included sizing with the aid of an optical scale, a serious source of experimental error.

The data obtained has been analyzed in terms of the current model (Ref. 1). In this model the characteristic force of adhesion F_o, corresponding to a removal fraction of 0.5, is given by:

$$F_o = k_o d \tag{1}$$

or the characteristic acceleration is given by:

$$a_o = \frac{6k_o}{\pi d^2 \rho} \tag{2}$$

where d is the diameter and ρ is the density of the particle and k_o is a constant to be determined.

The actual adhesion force F of a given particle of diameter d is assumed to be distributed according to a log-normal distribution in k. The parameters of the model are the mean value of log k equal to log k_o and noted as m and the standard deviation σ_r. The removal fraction for a group of particles of size d is then the probability that the applied force F* (or the applied acceleration a*) exceeds the adhesion force F (or the acceleration a), where the latter is distributed as discussed. This probability is written:

$$P(F^* > F) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{(\log k^* - m)/\sigma_r} dt \exp(-t^2/2) \tag{3}$$

where

$$a^* = F^*/d = \pi d^2 \rho a^*/6 \quad . \tag{4}$$

The results of these preliminary fits to the data are summarized in Table 4-A.1. The following conclusions may be drawn: (1) Glass beads as expected do not adhere as well as dust; (2) There is no statistical difference between adhesion at ambient pressure and at 10^{-5} mm Hg; (3) Dust may adhere better to glass at ambient pressure and at 10^{-5} mm Hg than to aluminum; (4) However, the adhesion of dust to glass is lower at 10 mm Hg pressure than of dust to aluminum and represents the only evidence for a pressure dependence.

Table 4-A.1. Particle Adhesion Results, Derived Values for m and σ_r*

Adhesion Case	At Ambient Pressure	At 10 mm Hg	At 10^{-5} mm Hg
Glass Beads on Glass	-1.73, 0.230	—	-1.59, 0.262
Dust on Glass	-1.15, 0.250	-1.51, 0.322	-1.19, 0.220
Glass Beads on Al	-2.10, 0.065	—	-2.12, 0.065
Dust on Al	-1.44, 0.265	-1.36, 0.270	-1.40, 0.245

*Units of m, σ_r such that 10^m and $10^{\sigma r}$ is in N/m.

A sample of the data, the six cases for 20 μm dust, along with the model curves are shown in Figs. 4-A.3 and 4-A.4. It should be noted that the curves represent a fit to all of the data for particle sizes ranging from 7.5 μm to 100 μm. Better fits for a particular size may of course be obtained.

4.1.2.2 Dynamic Release Mechanisms. During this reporting period the meteroid impact/surface response computer code has been run for the high temperature and the low temperature reusable surface insulation which comprise most of the orbiter surface. Results were obtained for the ensemble of meteroid masses.

4.1.2.3 Particle Transport Analysis. The overall system design for the STS orbiter recontamination is being contoured to conform to the previous strategy for Viking-type missions. Additionally, streamlining the main computer program down to a skeletal structure calling a large number of specialty subroutines which will make the overall computer code more transparent and efficient is an

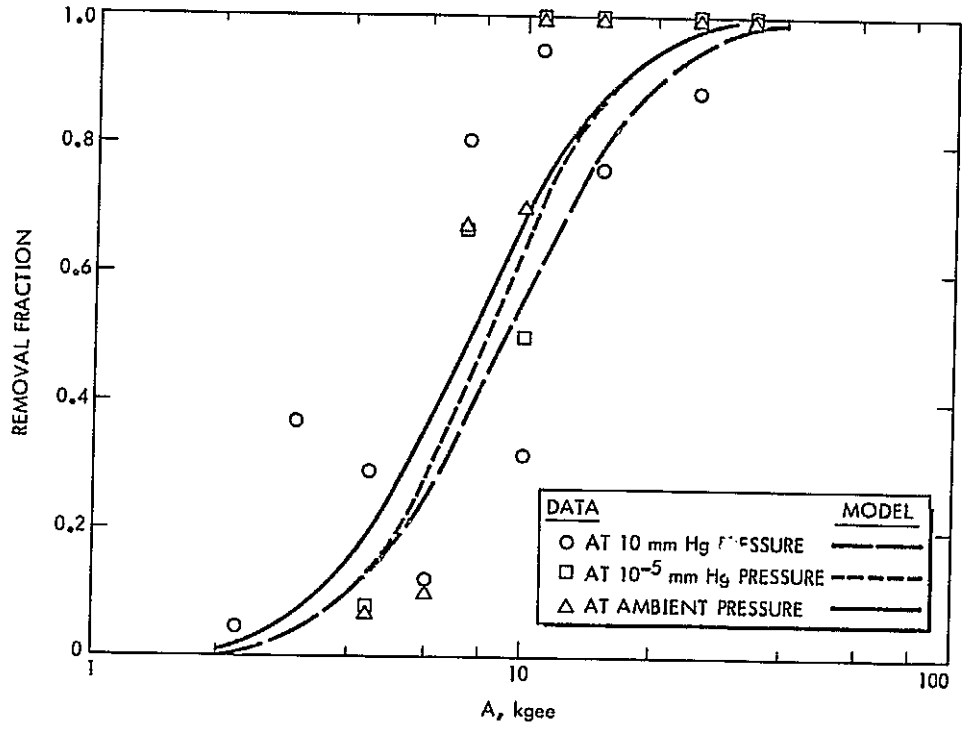

Fig. 4-A.3. Adhesion data and model for 20 μm dust on aluminum substrate

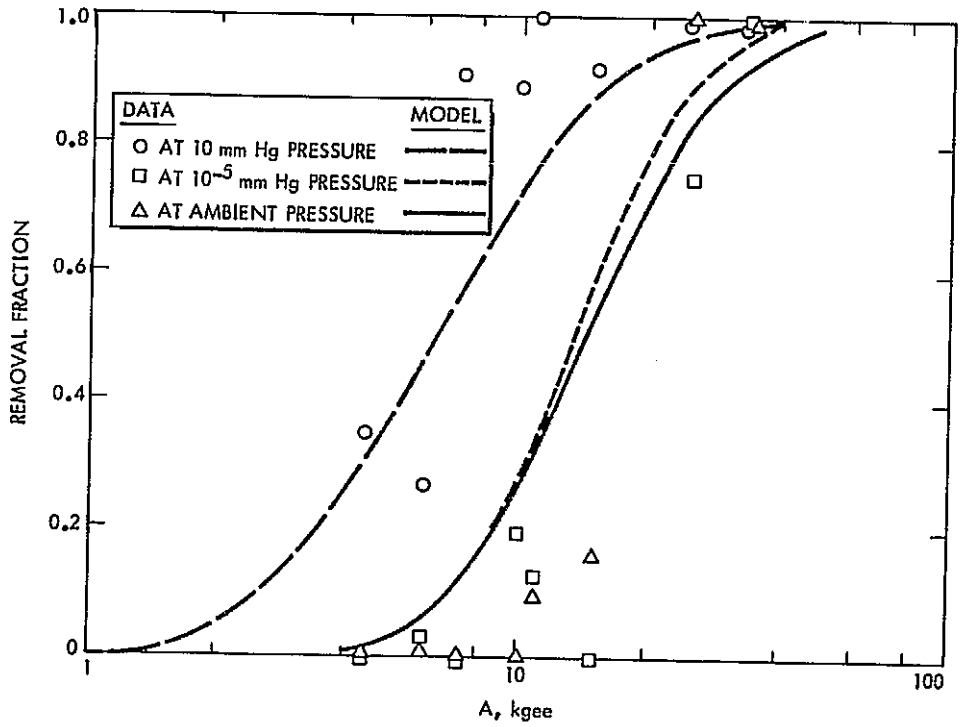

Fig. 4-A.4. Adhesion data and model for 20 μm dust on glass substrate

ongoing developmental objective. As described in Ref. 3, the new geometry of the STS orbiter and payload volume are now being encoded. The effects of the altered dimensional and orientational characteristics are being incorporated within many different levels of the transport calculation. During this report period, several computer subroutines have been revised to be consistent with the new geometry.

The geometric model (Figs. 4-A.5 and 4-A.6) incorporates the elliptical surface

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$$

to model the main fuselage of the STS. The lines:

$$L_1 \qquad y = L(1,1)\,x + L(1,2)$$

$$L_2 \qquad y = L(2,1)\,x + L(2,2)$$

$$L_3 \qquad y = L(3,1)\,x + L(3,2)$$

$$L_4 \qquad y = L(4,1)\,x + L(4,2)$$

bounding the planar pieces W_1, W_2 describe the wings. Two more lines

$$L_5 \qquad z = L(5,1)\,x + L(5,2)$$

$$L_6 \qquad z = L(6,1)\,x + L(6,2)$$

describe the tail section T.

During this reporting period several new computer subroutines have been encoded and are now debugged.

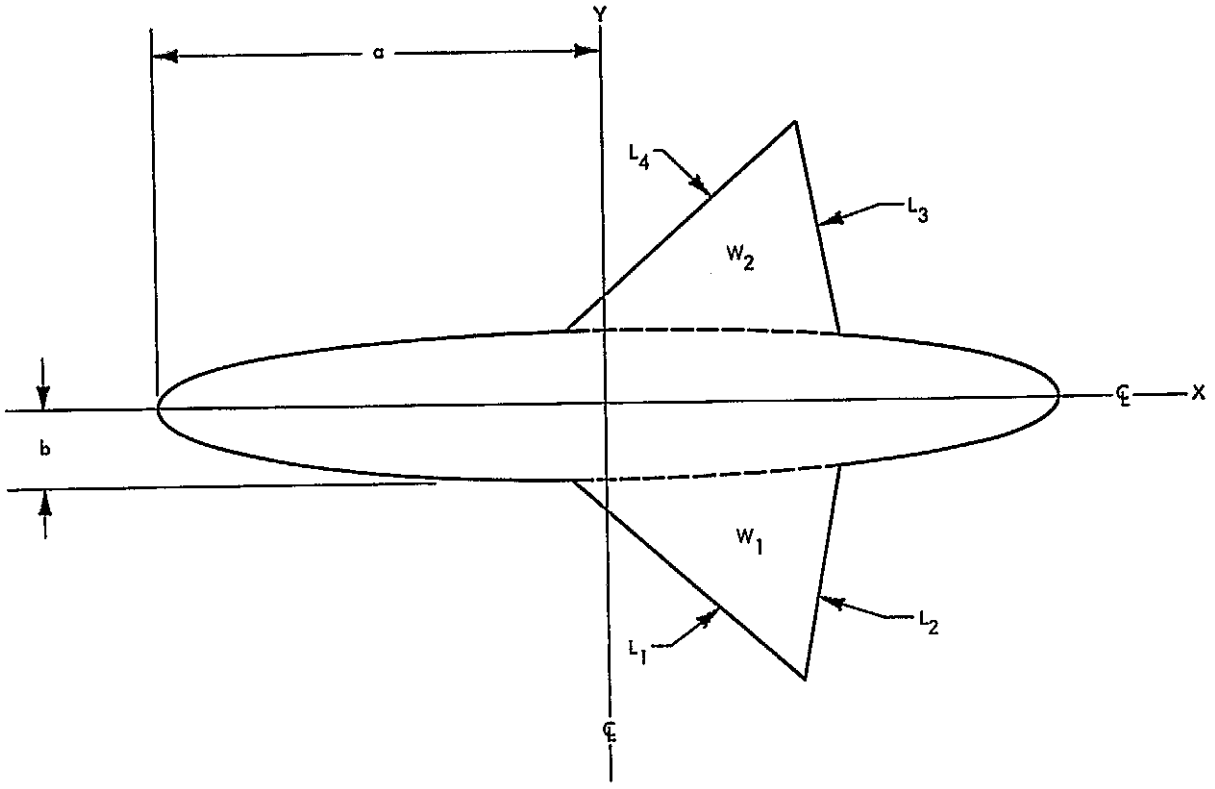

Fig. 4-A.5. Top view STS geometric model

CHKHIT and WGHIT are the subroutines which take a particle with an arbitrary trajectory relative to the spacecraft (S/C) through an incremental step in its dynamic trajectory to determine whether it impacts the surface of the S/C body or the wing/tail section. Provision is made at the same time to segregate those particle histories which traverse within the payload volume.

SCPLOT gives a graphical display of the S/C surface with some of the equipotential surfaces and the associated magnitude of the electric field on that surface. At the same time it checks the consistency of the S/C geometry.

SHADE, for a given sun vector, determines at each incremental trajectory position whether: the particle is in the sun or shade side of the earth, and the particle is in the sun or shade side of the STS orbiter.

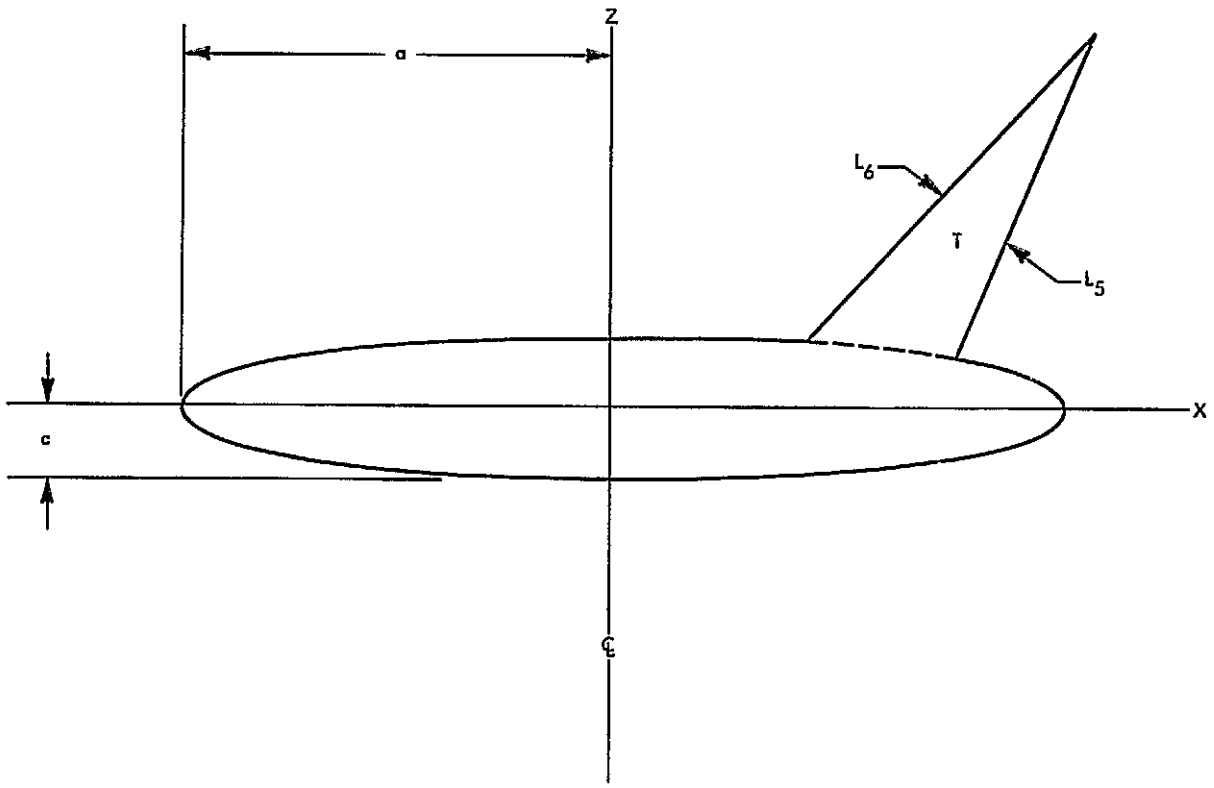

Fig. 4-A.6. Side view STS geometric model

EFDPTL calculates the electric field and potential acting on a charged particle near the STS orbiter using the elliptical co-ordinate system which is natural to the physical model. For a distance from the surface larger than 5 debye lengths, a simplified algorithm evaluates the electric field as due to a spherical craft. The development of EFDPTL required the introduction of several other computer subroutines to make EFDPTL usable. Subroutine AORDER was developed to order the elliptic co-ordinates from largest to smallest so that subroutine RETRAN which transforms from rectangular to ellipsoidal co-ordinates functioned properly. Subroutine EPTRAN which transforms from ellipsoidal to rectangular co-ordinates was written to allow a range of input parameters to be analyzed in ellipsoidal co-ordinates. EFDPTL also required the development of an algorithm to find the normal to the surface at any point in order to evaluate the distance to the orbiter surface.

4.1.2.4 Sources of New Particles. During the previous period, several sources of new free particulates which could be more important than the dynamic release of surface contaminant particles were identified. The new particle processes under consideration are backsplash from meteroid impact (the crater and the meteroid material), flaking from edges and surfaces due to thermal gradients and wear, and the abrasion of aluminum - aluminum moving interfaces (e.g., separation joints and deployment hinges).

Two models for meteroid backsplash have been developed during this reporting period. These models have been incorporated into computer codes which yield number distributions in mass and velocity for specified impact parameters and assumed properties of the principal orbiter surface materials. Data on meteroid impact on these materials will be required to choose between the two models and to refine the better one.

An analysis of the abrasion of aluminum-aluminum interfaces has been conducted. This mechanism is clearly negligible compared to meteroid impact as a result of this analysis.

4.1.3 Future Activities

Under particle adhesion the new data will be extensively analyzed to yield a revised model.

The bulk of the work remaining is in the particle transport analysis. The remaining geometry-dependent codes will be updated. The effect of the transient electric field generated at the sun-shade interface of the orbit will be evaluated. The S/C and particle charging codes will be revised to include the ionospheric radiation belts effect. The co-ordinate transforming algorithm to connect the STS orbiter coordinate system to the Earth orbit coordinate system will be developed.

When data becomes available, the model for the meteroid backsplash mechanism as a source of new particles will be revised.

4.1.4 References

1. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July - December 1973, JPL Doc. 900-655, p. 3-1, 1974.

2. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January - 30 June 1975, JPL Doc. 900-715, p. 4-13 ff., 1975.

3. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July - 30 December 1975, JPL Doc. 900-732, p. 3-3 ff., 1976.

SECTION V

CONTAMINATION MEASUREMENT AND CONTROL
(NASA No. 193-58-63-02)

Contents	Title and Related Personnel
Subtask A Para. 5.1	Improved Biosampling Technology
	Cognizance: R. Koukol
	Associate Personnel: G. Simko (Bionetics) W. Neiderheiser (Bionetics)
Subtask B Para. 5.2	Evaluation of Vacuum/Heat Sterilization
	Cognizance: A. Irons
	Associate Personnel: G. Simko (Bionetics)
Subtask C Para. 5.3	Bio-Detection Techniques
	Cognizance: J. Jacobs
	Associate Personnel: J. Barengoltz C. Myers I. Ickovits (Bionetics)

SECTION V

CONTAMINATION MEASUREMENT AND CONTROL

5.1 IMPROVED BIOSAMPLING TECHNOLOGY

5.1.1 Subtask A Introduction

The objective of this subtask is to develop an improved biosampling technique for use on spacecraft hardware. Experience in past flight projects (Viking, Mariner 9, Mariner 6 and 7) indicates that a faster and more efficient sampling system would be beneficial to a flight project. The currently used swab-rinse technique allows data reproducibility; however, it requires approximately 8 hours of spacecraft time for each bioassay to be performed. The swab-rinse technique is limited to 12.9 cm^2 (4 $in.^2$) with the result that most (90%) of the data points are zero. According to Efron[1] this percent of zero samples gives a "very unstable estimator of the true population mean in such conditions." If a biosampling device could sample up to 92.9 cm^2 (1 $ft.^2$), this would likely reduce the number and percentage of "zero" samples and give a better mean estimation. Additionally, the swab-rinse technique has been found to be only 30% to 50% efficient in recovering microorganisms.

Previous work dealing with physical removal of spacecraft microbial burden indicates that a jet deflection technique utilizing a liquid under pressure is very efficient at removing particulates and it appears that the sample size can be at least 92.9 cm^2. The use of a liquid recovery medium should eliminate the dessication of organisms and sonication of apparatus hardware associated with dry vacuum recovery systems. Dry vacuum sampling and recovery systems trap microorganisms within the sampling device itself necessitating the sonicating of the sampling device to achieve a total biological count. Additionally, many types of microorganisms cannot survive prolonged exposure to the high airflow associated with such sampling devices and their concomitant recovery systems.

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[1] Analysis of Microbial Burden Data. Planetary Quarantine Annual Review of Space Technology and Research, 1973, 900-597.

5.1.2 Approach

This subtask is designed to examine the jet deflection technique as a removal system and determine its feasibility and application as a spacecraft hardware biosampling device.

Several possible jet deflection techniques have been studied. After theoretical feasibility studies were performed, two techniques were selected for further study. These are the oscillating rod and hydro-aerodynamic (wet vacuum) sampling techniques. The oscillating rod (Figure 5-A.1) technique has been previously described.[2]

The hydro-aerodynamic or wet vacuum sampling technique (Figure 5-A.2) cleans by sweeping the surface with a thin but coherent sheet of liquid produced by impinging a slant baffle with a metered and also coherent jet of liquid (Figure 5-A.3). The liquid sheet (a film) passes through a narrow slot formed between the surface and the lip of the vacuum nozzle. The air entering the nozzle plenum chamber from all sides between the surface and the nozzle lip forms a pair of counter-rotating vortices (Figure 5-A.4), the cores of which coincide with the loci of the hydraulic jump of the film flow. The effect is that the film rises out of the boundary layer where it is efficiently entrained into the updraft of the vortex flow. The sample surface is dry after passing the vortex core.

Activities involved in this study include conceptual design and testing of a biosampling system, determination of potential sampling liquids, and development of a collection device. The design of the system and the selection of sampling liquids is critical to the application of the system for bioremoval and detection as they must be completely compatible with spacecraft hardware to eliminate any possibility of damage.

The collection device must employ a technique which traps the microorganisms without the dessication normally associated with vacuum bio-sampling devices.

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[2] Planetary Quarantine Semi-Annual Review, Space Research and Technology, September, 1974, 900-675.

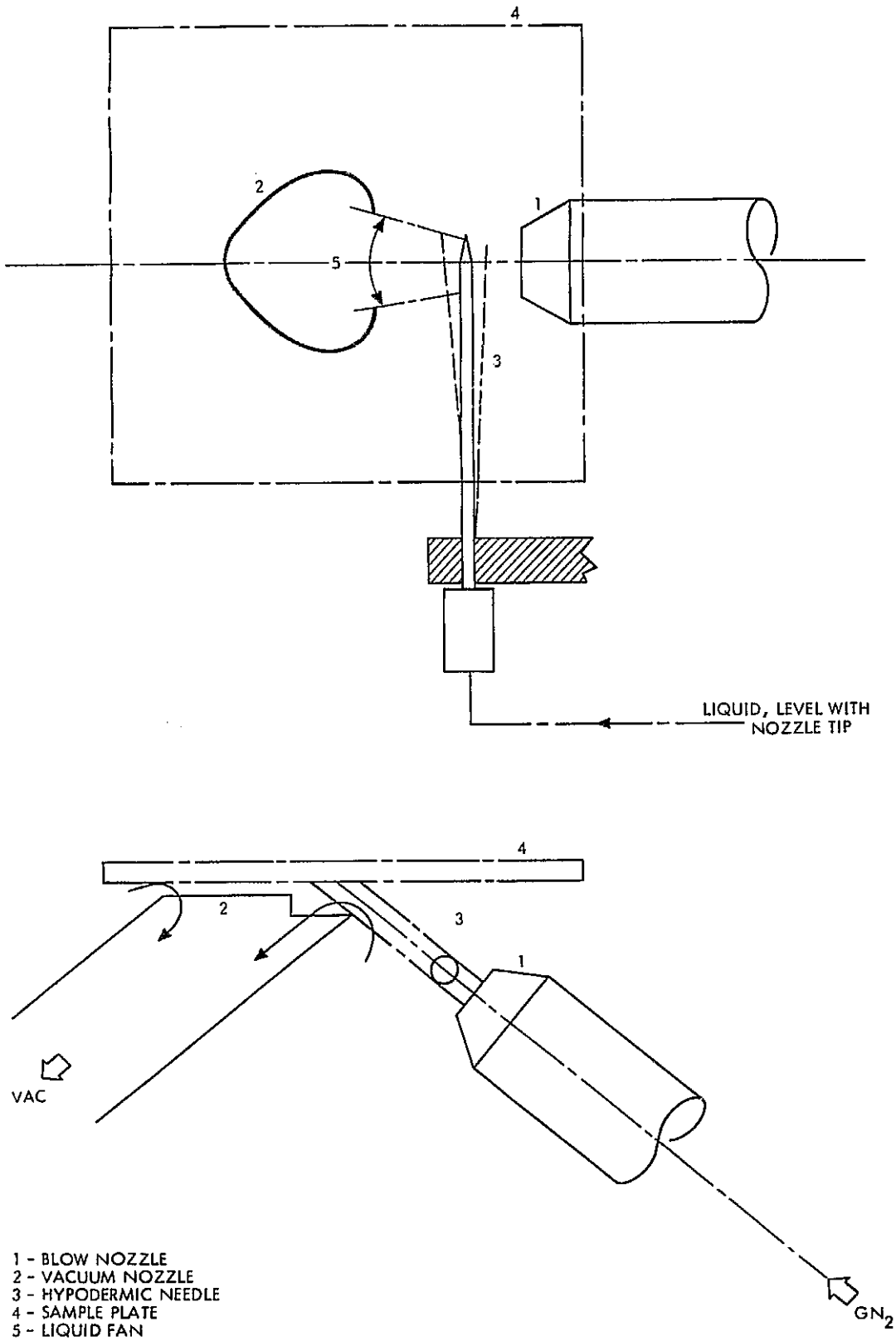

Fig. 5-A.1. Schematic of oscillating rod set up

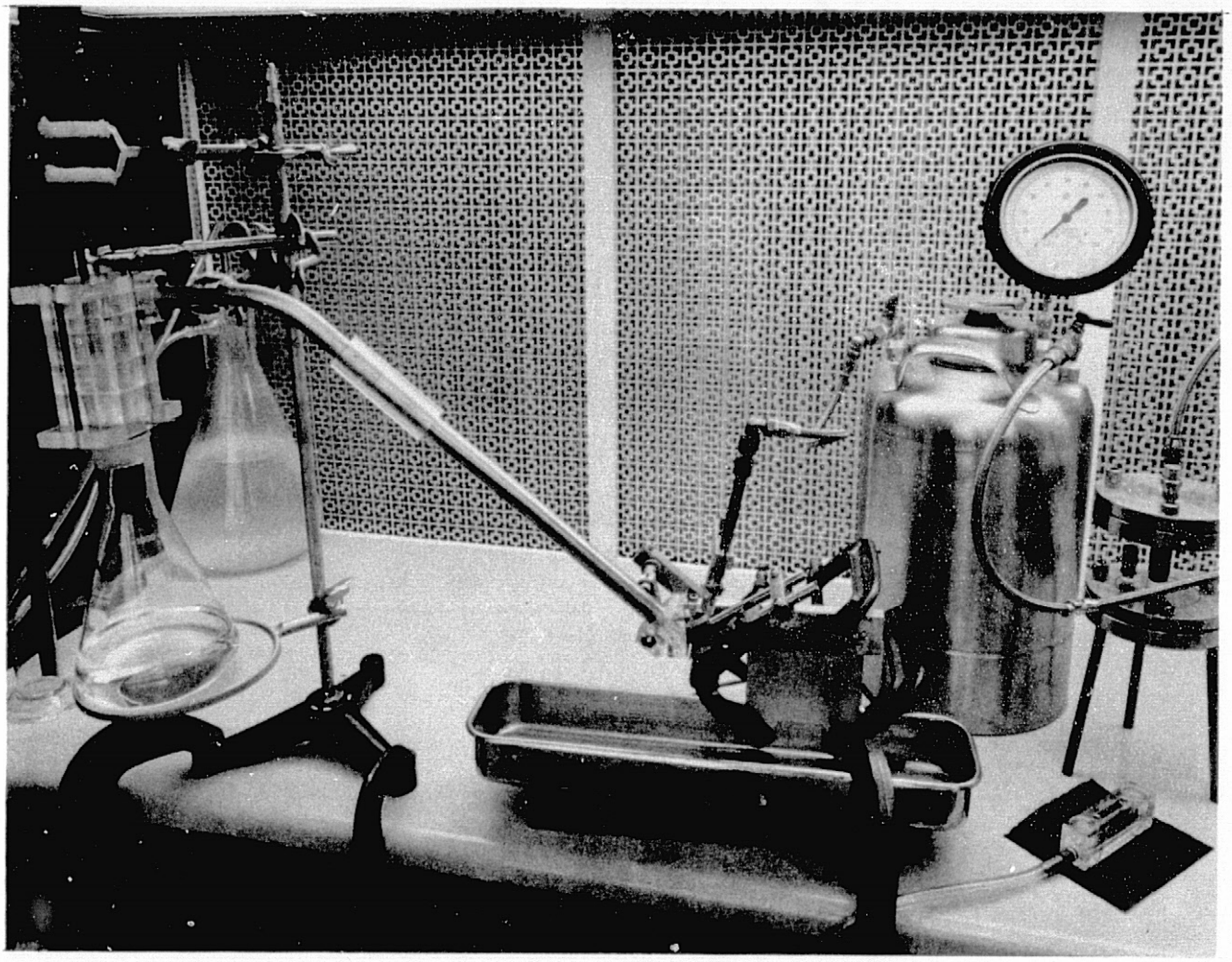

Fig. 5-A.2. Hydro-aerodynic bio-sampling test setup

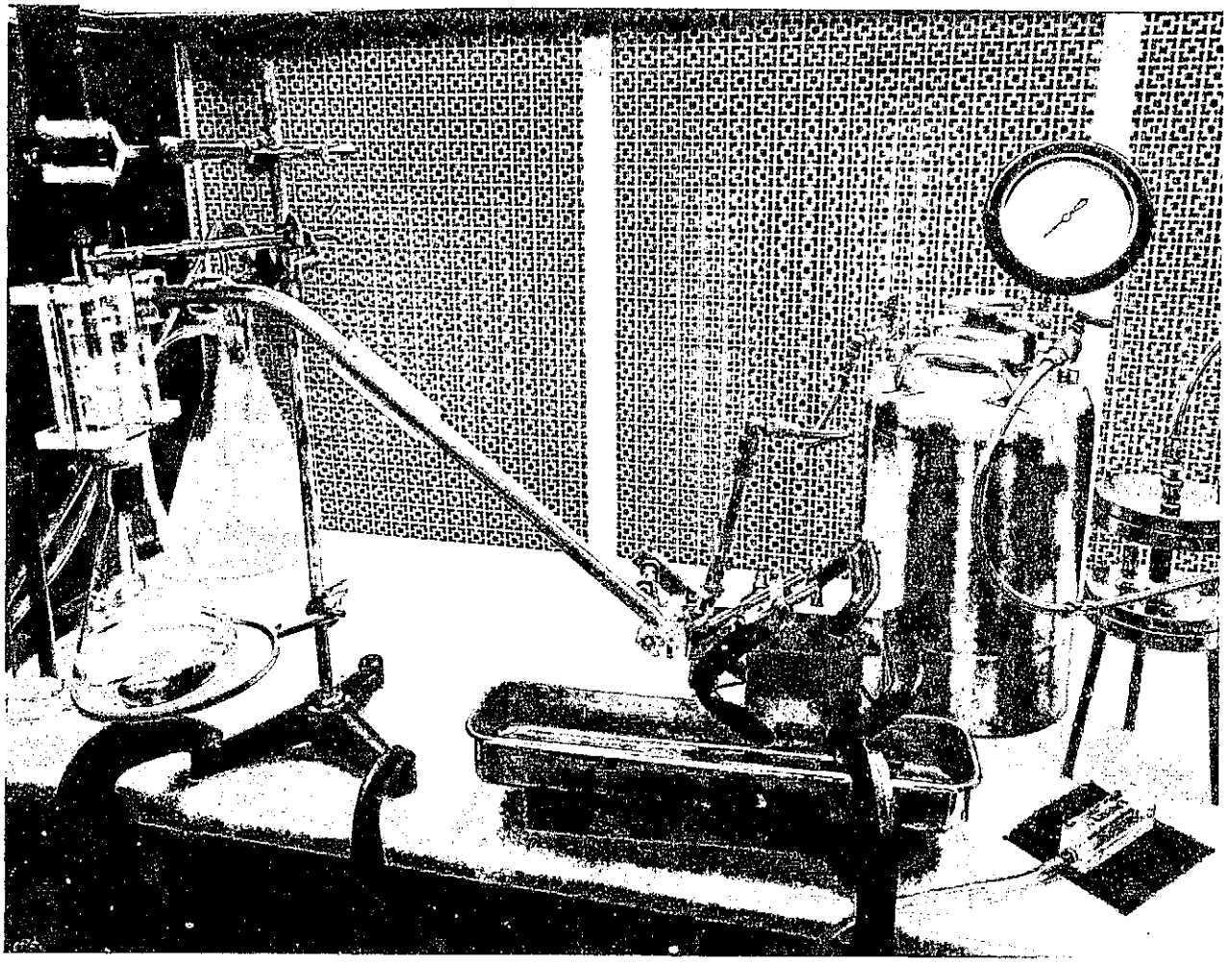

Fig. 5-A.2. Hydro-aerodynic bio-sampling test setup

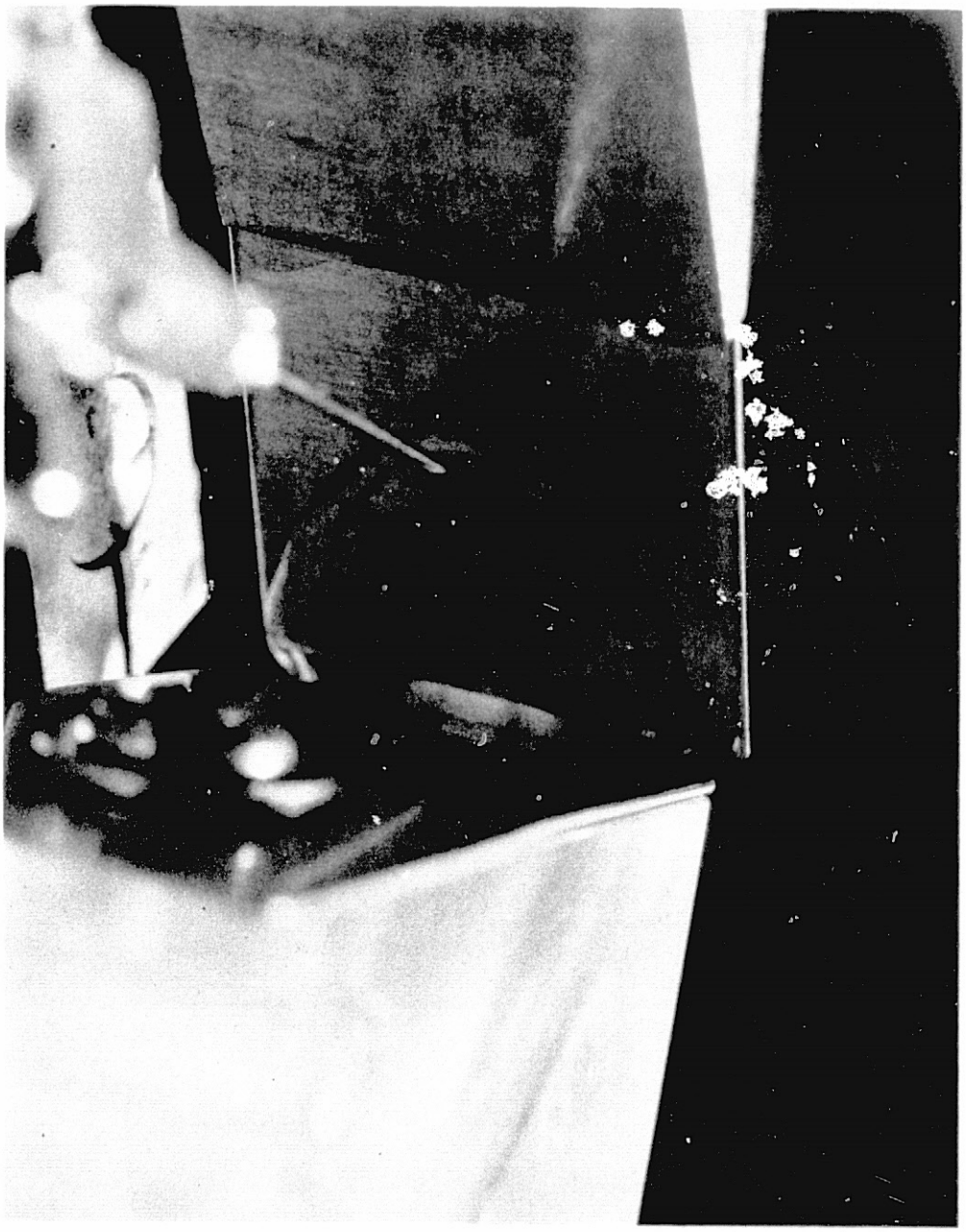

Fig. 5-A.3. Liquid sheet formed by impinging jet of liquid on slant baffle

Fig. 5-A.4. Counter rotating vortices formed by uptake of sampling liquid into vacuum system

5.1.3 Significant Accomplishments

Activities at JPL include the determination of sampling efficiency, potential sampling liquids and development of a collection device. Recovery efficiency studies have also been initiated. Sampling efficiency, for purposes of this study, is defined as that percentage of the microorganisms removed during the biosampling process. Recovery efficiency is defined as the percentage of the microorganisms trapped and reacquired by the recovery system.

In initial biological testing at JPL, using several population levels of Bacillus subtilis var. niger spores, the oscillating rod sampling device established a sampling efficiency of 51 to 83 percent. The oscillating rod concept was abandoned because of difficulties encountered in producing rod resonance and repeatable spray pattern.

To improve overall sampling efficiency significant effort has been spent on the design and fabrication of the hydro-aerodynamic sampling device. In tests comparable to those on the oscillating rod, using the hydro-aerodynamic sampling technique, the data indicated a sampling efficiency in excess of 95 percent as shown in Table 5-A.1.

Table 5-A.1. Comparison of oscillating rod and hydro-aerodynic sampling techniques

	Removal (%)	Recovery (%)
Oscillating rod	51-83	--
Hydro-aerodynic system	>95	83-90
40 lb. pressure 150 mm Hg vacuum (average pressure) Bacillus subtilis var. niger		

One problem in developing a feasible spacecraft biosampling device is to determine acceptable and practical liquids for use as a recovery medium. Discussion with several spacecraft engineers has limited acceptable liquids to distilled water and alcohols. No other liquids are allowed within the assembly facilities.

A recovery or collection device utilizing a cyclonic inertial separator (Figure 5-A. 5) has been developed. This is a method of recovering organisms dislodged by the removal system without the dessication normally associated with vacuum biosampling devices as noted by the above results.

5.1.4 Future Activities

The hydro-aerodyanamic sampling technique will continue to be evaluated as a sampling method based on a series of biological tests. The tests will be divided into several phases to isolate various system peculiarities from each other, such as the sampling or removal efficiency of the device, the overall recovery efficiency, and interior contamination and the ability of the system to clean itself.

The sampling or removal efficiency of the system has been determined as stated above. Preliminary data in the area of recovery efficiency using B. subtilis var. niger is very promising. Future work will be performed to evaluate recovery efficiency by varying the microorganism population of the sample. Study will be initiated into the area of system contamination and self-cleaning. Tests will be conducted to determine the extent of purging with sterile sampling liquid necessary to insure acceptably low levels of contamination in the system between samples.

Tests will also be conducted to determine the application of the sampling technique for use with vegetative (non-spore forming) and naturally occurring (fallout) organisms. These tests will be performed in a manner similar to those performed using B. subtilis var. niger.

Completion of this biological testing program should define the capabilities and applications of the sampling device as a practical biodetection tool. After feasibility of the system as a spacecraft biodetection device has been established, a prototype biosampling system will be developed, which will be compatible with spacecraft hardware. Biodetection studies using the prototype device will then be conducted to determine the sampling and recovery efficiencies. These tests will be conducted using spores, vegetative, and naturally occurring microorganism populations.

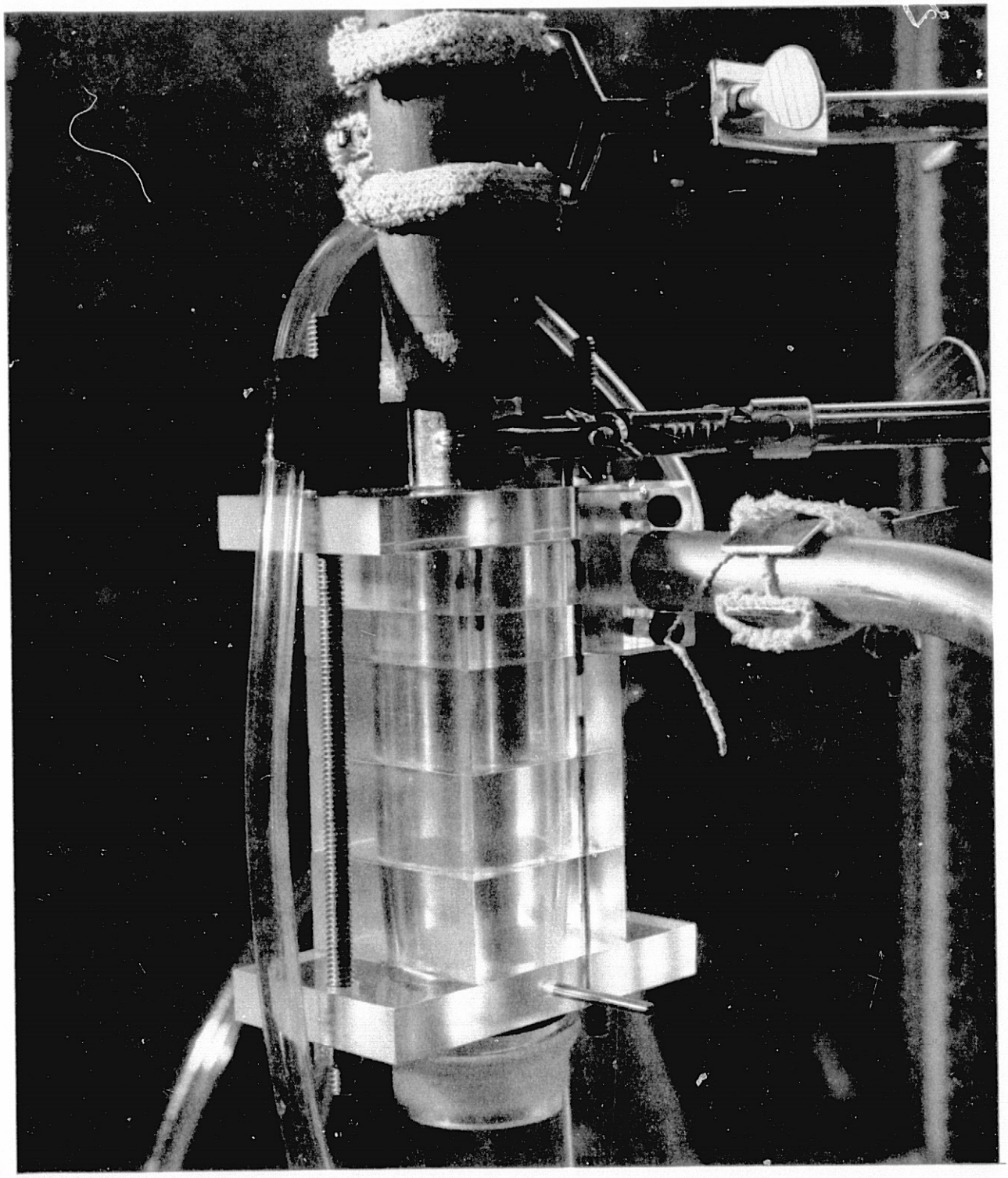

Fig. 5-A.5. Cyclonic inertial separator

5.2 EVALUATION OF VACUUM/HEAT STERILIZATION

5.2.1 Subtask B Introduction

One aspect of previous vacuum/heat studies (Ref. 1) was to look into the findings of Davis, Silverman, and Keller (Ref. 2). They reported better survival of Bacilius subtilis var. niger spores at atmospheric pressure than in a vacuum of 10^{-8} to 10^{-10} torr at both 60° and 90°C. The apparent enhancernent of dry heat lethality by vacuum conditions may provide an improved spacecraft decontamination/sterilization process. This revised process could be more compatible with spacecraft hardware, increase spacecraft reliability and possibly increase the probability of achieving a specified level of sterility.

Another element of these studies stems from the information obtained from tests performed at JPL and reported in an earlier JPL Semi-Annual Review (Ref. 3). As a result of these tests it has been theorized that an organism's ability to withstand a given substrate temperature depends on the chamber material and color (infrared reflectivity) and chamber wall temperature as well. This warranted the fabrication of a new test fixture (Figure 5-B.1). The new test fixture consists of a closely-controlled substrate temperature and a "chamber wall" with independent temperature control. Its geometric design lends itself to good thermal flux analysis. The fixture can be used in existing vacuum chambers to produce the required test conditions regardless of the chamber wall temperature or surface color. Auxiliary heat exchangers utilizing heated or cooled gases may be employed with the new fixture configuration to augment electrical resistance heaters and permit reduction of the time to reach substrate test temperature and return to ambient. The reduction of this ramp time represents a significant reduction in a source of experimental error, especially for short exposures. The primary objectives of this task are to clarify and define the effects of chamber wall temperature, infrared reflectivity and other parameters on the sterilization efficiency of dry heat applied concurrently with vacuum or atmospheric pressure as well as to develop a better understanding of the vacuum/heat synergism which decreases the heat resistance of spores.

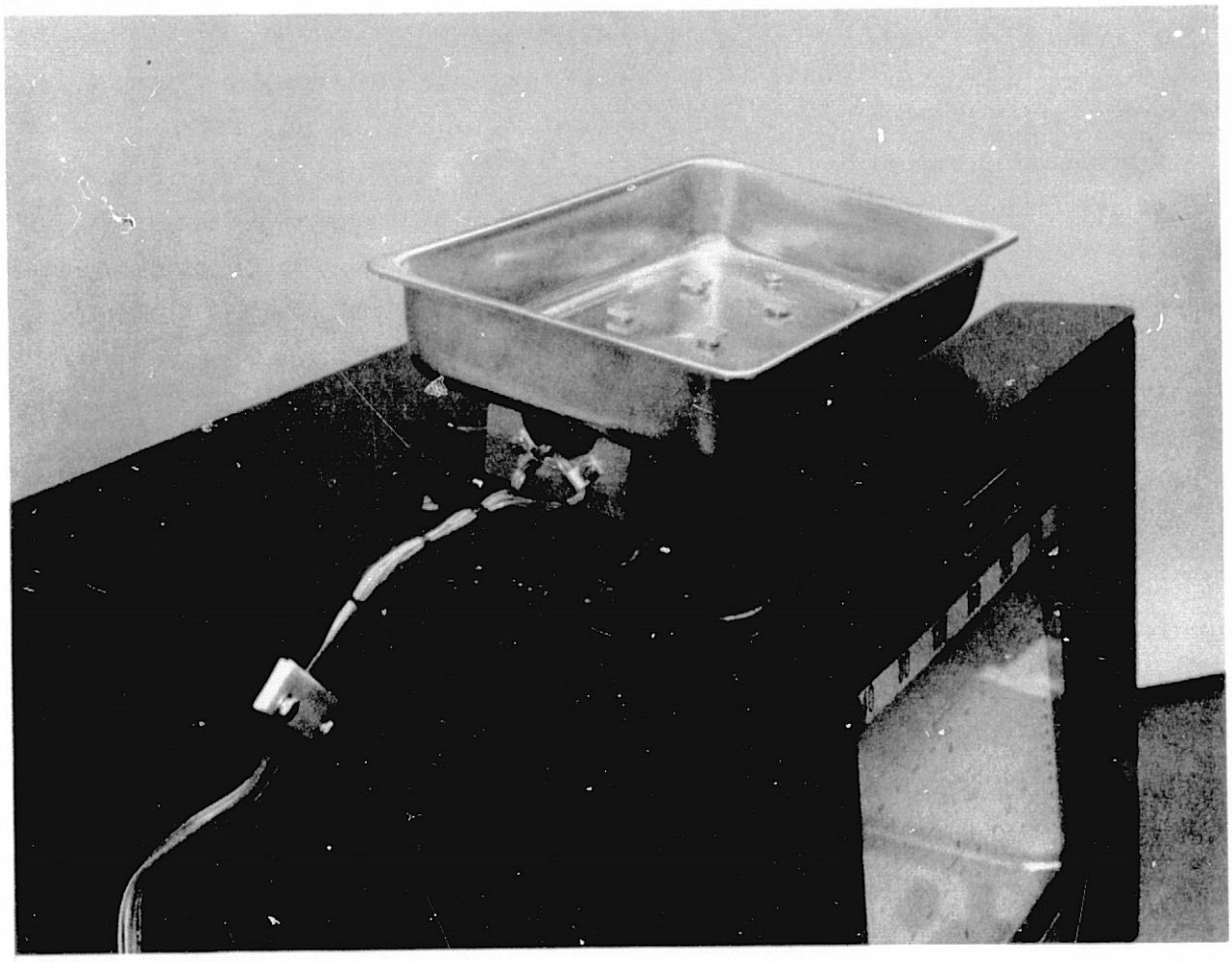

Fig. 5-B.1. Vacuum/heat test fixture

5.2.2 Approach

A two-phased approach was taken in order to better present the varying test conditions and also to permit presentation of the data in a more clear and concise manner.

5.2.2.1 Phase I. Phase I constitutes the work performed on Bacillus subtilis var. niger (BSN) spores. The substrate temperature was 80°C with the cryo-plate temperature at -185°C, -35°C, 23°C, 80°C and 100°C. Both the reflective (shiny) and non-reflective (black) sides of the cryo-plate were used. Exposure duration for the various test conditions were 2, 16, 24, 48 hrs under a vacuum of 10^{-5} torr. Several tests were conducted utilizing the heat source in the shroud of the vacuum chamber. In these tests all three elements (substrate, cryo-plate and shroud) were heated; when the desired temperature was reached the substrate and cryo-plate heat source was cut off and the temperature was maintained by the shroud only. Tests were conducted with the shroud at 80°C and 100°C.

Table 5-B.1 shows the cumulative results of all the various parameters studied under vacuum. The effect on the survival of B. subtilis spores when utilizing the shroud as the heat source as opposed to heating the substrate and cryo-plate directly is shown on Fig. 5-B.2.

The effect of wall temperature on the survival of B. subtilis spores is shown in Fig. 5-B.3. The substrate was maintained at 80°C at a pressure of 10^{-5} torr. The cryo-plate had its shiny side facing the substrate.

The effect of chamber wall reflectivity at a substrate temperature of 80°C and a pressure of 10^{-5} torr, on the survival of B. subtilis spores, is shown in Fig. 5-B.4.

The present studies shows none of the previously observed effects of chamber wall infrared reflectivity and temperature, on the thermal death of B. subtilis spores placed on a substrate at a constant temperature (80°C) and a pressure of 10^{-5} torr. It is believed that the apparent "chamber wall" effect observed in previous studies was an artifact due to some undetected and uncontrolled factor or factors among the various vacuum test systems. Present data, utilizing the new test fixture, indicates that highly reproducible results may be obtained with, good physical control.

Table 5-B.1. Effect of test conditions on Bacillus Subtilis var. niger in vacuum

Duration, hrs	Temperature, °C			Reflectivity of Cryoplate		Survival Fraction
	Substrate	Cryoplate	Shroud	Shiny	Black	
2	80	-185	-		X	0.48
16	80	-185	-		X	-
24	80	-185	-		X	0.0046
48	80	-185	-		X	0.000058
2	80	23	-		X	-
16	80	23	-		X	0.035
24	80	23	-		X	0.0044
48	80	23	-		X	0.000067
2	80	23	-	X		0.44
16	80	23	-	X		0.02
24	80	23	-	X		0.0052
48	80	23	-	X		-
2	80	80	80	X		0.33
16	80	80	80	X		0.025
24	80	80	80	X		0.0042
48	80	80	80	X		-
2	80	80	-	X		-
16	80	80	-	X		0.02
24	80	80	-	X		0.0048
48	80	80	-	X		-
2	100	100	100	X		0.0074
16	100	100	100	X		0
24	100	100	100	X		-
48	100	100	100	X		-

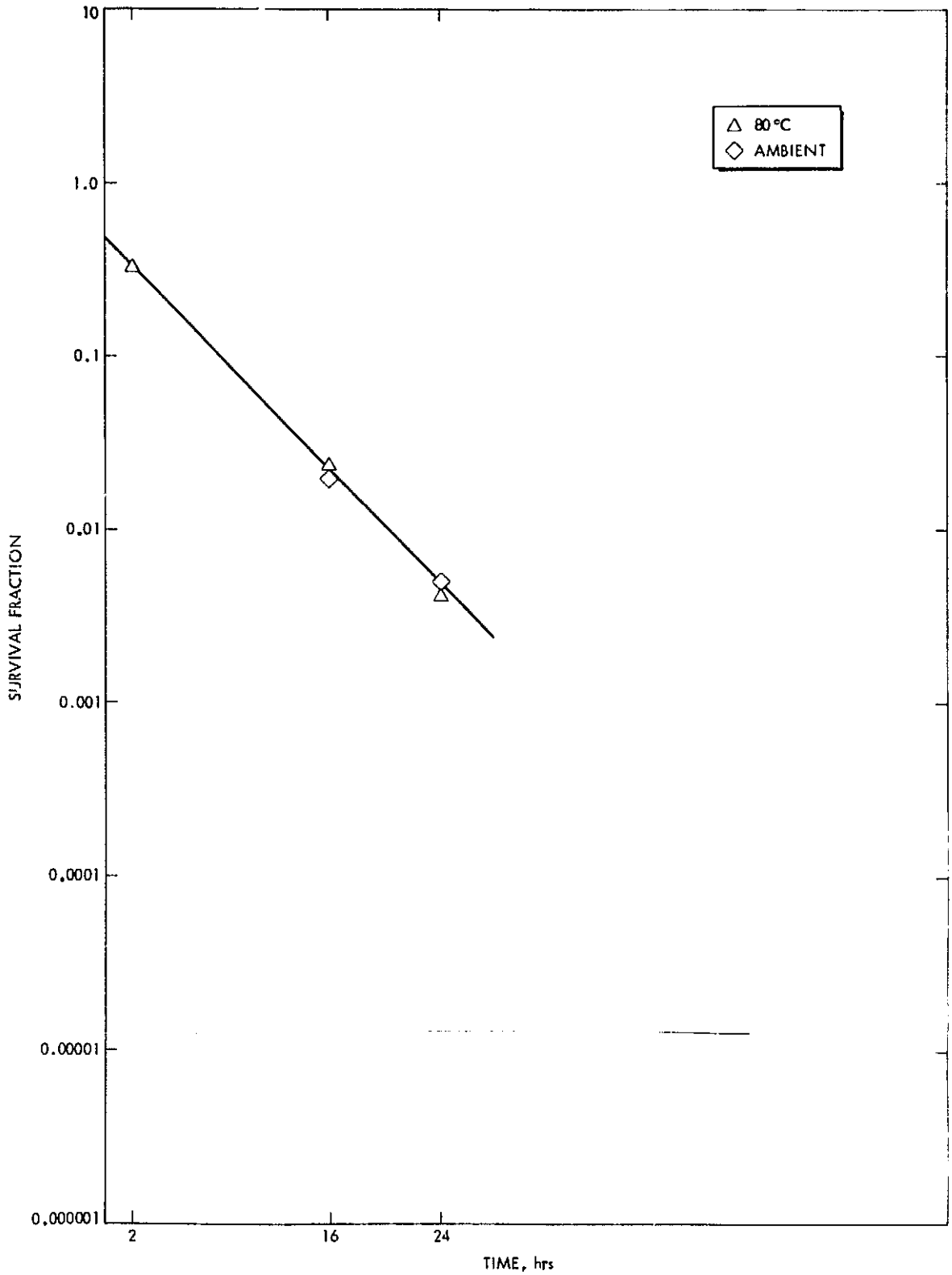

Fig. 5-B.2. Effect of shroud temperature

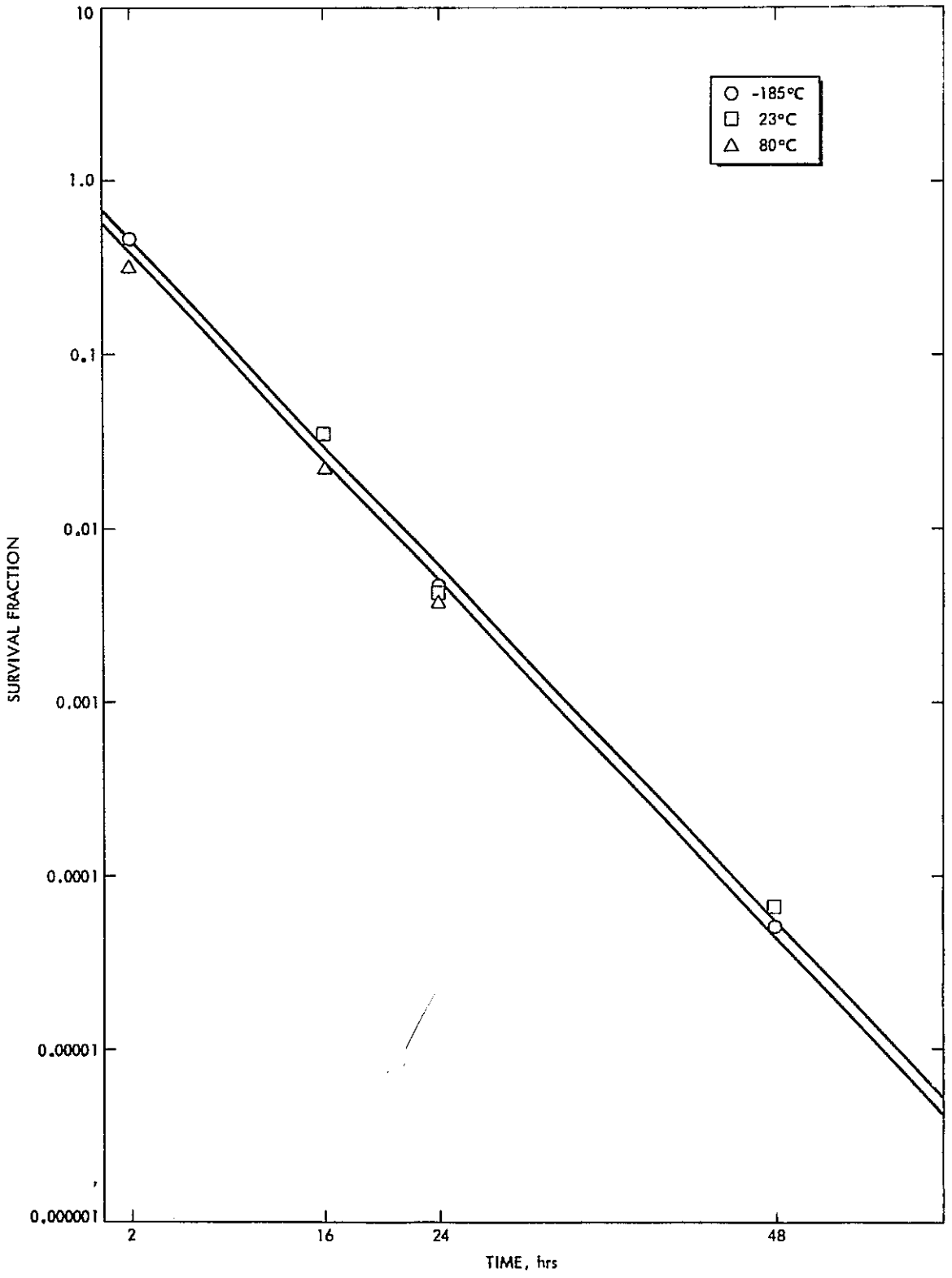

Fig. 5-B.3. Effect of wall temperature

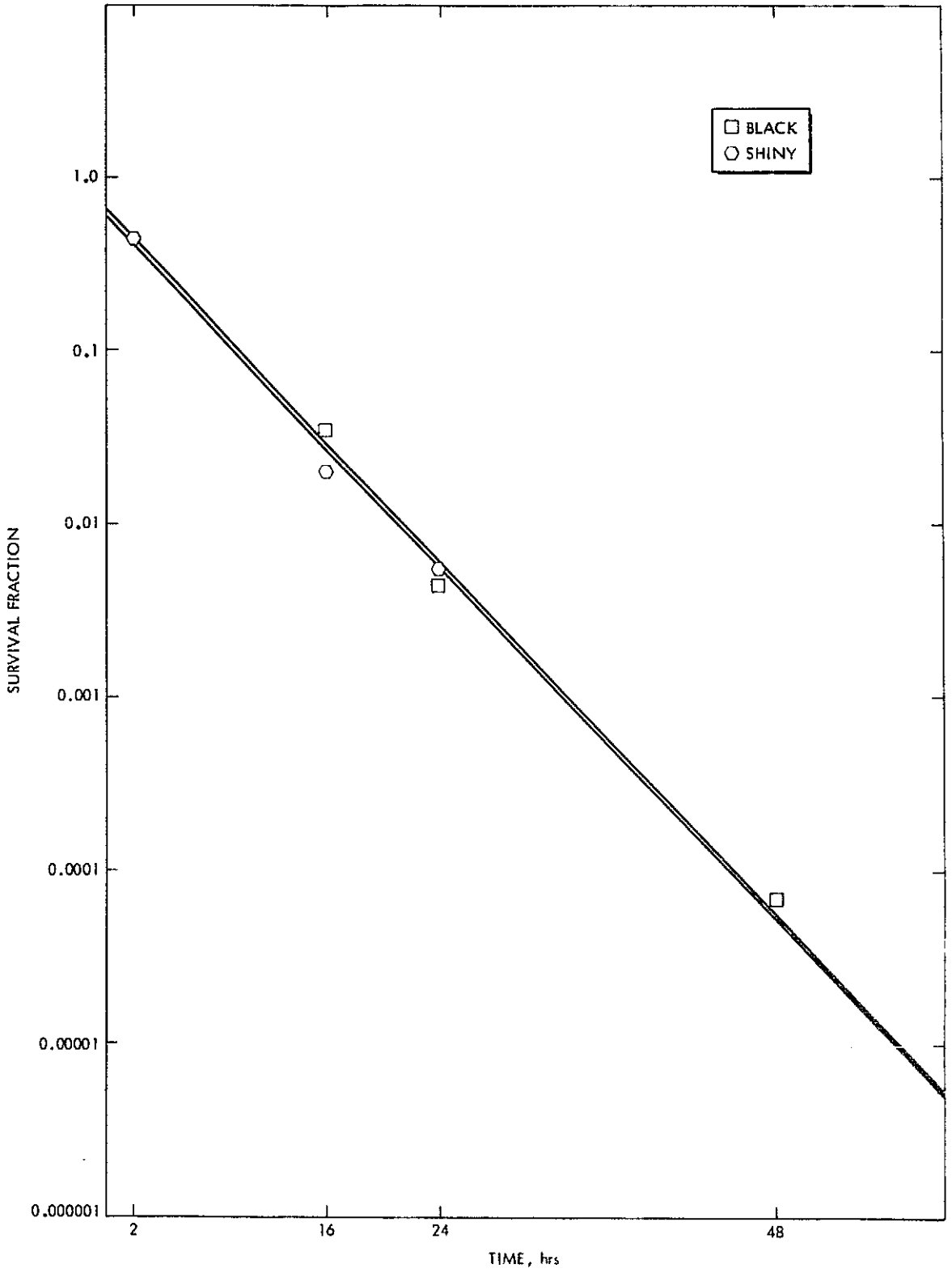

Fig. 5-B.4. Effect of wall reflectivity

The data also confirms previous results that indicate an enhanced lethality for bacterial spores in vacuum/dry heat compared to an atmospheric pressure environment. A comparison of the survival fractions for BSN spores at 100°C and 1 x 10^{-5} mm Hg to BSN spores at 100°C and one atmosphere of dry nitrogen shows a significant decrease in the typical exposure time for an order of magnitude reduction for the vacuum case (i.e., decrease in the absolute value of the inverse slope of the logarithm of survival fraction versus time).

5.2.2.2 Phase II. Phase II deals with continued work on B. subtilis spores; plus work on Bacillus brevis spores (a "hardy organism") and natural occurring organisms. The substrate Cryo-plate, and shroud temperature will be 80°C, 100°C, 113°C, 125°C for durations of 2, 16, 24, 48 hrs at both a vacuum of 10^{-5} torr and at ambient pressure with one atmosphere of dry nitrogen. The vacuum chamber shroud cannot be used as a heat source for ambient pressure runs at temperatures greater than 80°C due to heat loss from the chamber.

The Bacillus brevis spores were obtained from Dr. J.E. Campbell and A.L. Reyes of the Cincinnatti Ohio Food Research Laboratory, Bureau of Foods, Food and Drug Administration.

Table 5-B.2 shows the results to date of the effects of temperature on B. brevis spores under vacuum. Comparative ambient pressure runs have yet to be performed. Figure 5-B.5 shows the survival fraction curves for the above vacuum tests.

5.2.3 Future Activities

Complete Phase II utilizing spore cultures of B. subtilis and B. brevis as well as naturally occurring organisms. The naturally occurring organisms will not be cultured prior to vacuum/heat exposure. The body of survival fraction data obtained will be reduced into statistically valid survivor curves. Enhancement of lethality due to vacuum will also be analyzed.

Table 5-B.2. Effects of temperature on Bacillus brevis under vacuum

Organism	Duration, hrs	Temperature, °C	Pressure, torr	Survival Fraction
B. Brevis	2	100	10^{-5}	0.193
B. Brevis	16	100	10^{-5}	0.049
B. Brevis	24	100	10^{-5}	0.060
B. Brevis	48	100	10^{-5}	0.060
B. Brevis	2	113	10^{-5}	-
B. Brevis	16	113	10^{-5}	0.014
B. Brevis	24	113	10^{-5}	0.011
B. Brevis	48	113	10^{-5}	0.004
B. Brevis	2	125	10^{-5}	-
B. Brevis	16	125	10^{-5}	0.0007
B. Brevis	24	125	10^{-5}	0.00049
B. Brevis	48	125	10^{-5}	0/0000

5.2.4 Presentation

5.2.5 References

1. JPL, Planetary Quarantine Semi-Annual Review, JPL Document No. 900-732, pp. 4-4 to 8, 1976.

2. Davis, N. S., Silverman, G. J., and Keller, W. H., "Combined Effects of Ultrahigh Vacuum and Temperature on the Viability of Some Spores and Soil Organisms," Appl. Microbiol. Vol. II, pp. 202-210, 1963.

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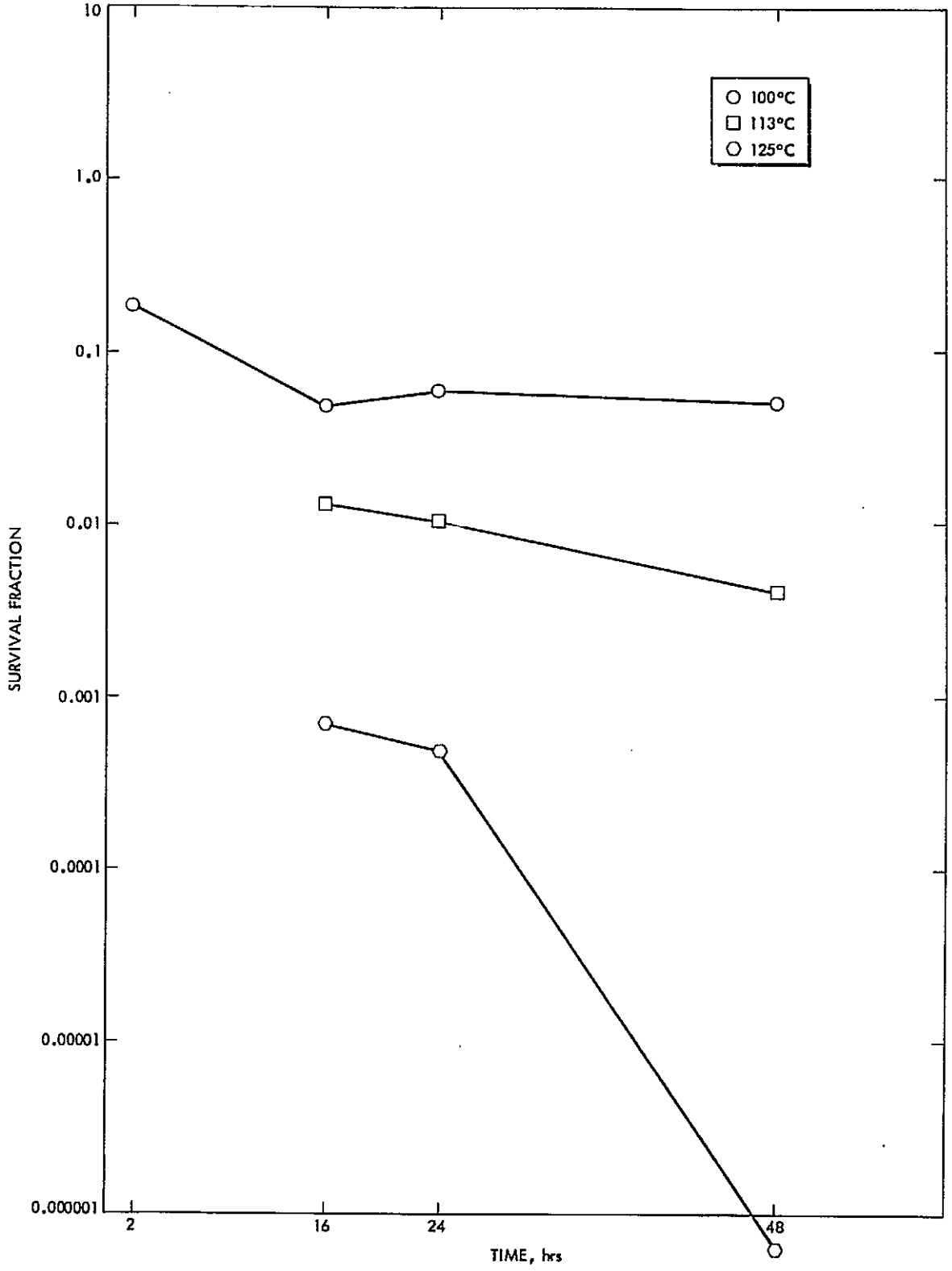

Fig. 5-B.5. Effects of temperature on B. brevis under vacuum

5.3 BIO-DETECTION TECHNIQUES

5.3.1 Subtask C Introduction

Present technology (ERTS/Landsat) allows remote detection of biosystems on a macroscopic scale, i.e., multispectral sensing of agricultural and forest crops as well as marine populations. It has been suggested that perhaps this same technology could be applied to microscopy and allow the detection of a microscopic biosystem such as microorganisms on a surface.

A procedure has been described by Casida (Ref. 1) for visual observation of bacteria, actinomycetes, fungi, protozoa, and other biosystems in masses of soil. This technique utilizes a microscope with reflected light, a broad band white light source and time lapse photography. Differentiation of microorganisms from soil minerals and organic particles was accomplished by color discrimination using color photography. The diffraction colors of in situ soil microorganisms corresponds with the green wavelength of 490 to 500 nm. Soil particles appear translucent, grey, brown or black.

It should be noted that Casida's observations did not require special sample preparation, and the observed characteristic wavelengths were most prominent during periods of low metabolic activity as demonstrated by comparison with organisms grown on agar blocks.

Identification of additional characteristics such as size, shape and response of microorganisms when subjected to monochromatic light may be necessary to differentiate between mineral particles and biosystems on spacecraft surfaces.

Kamentsky and Melamed (Ref. 2) demonstrated techniques used to measure multiple spectrophotometric properties of cells for differential classification. Lent and Nichols (Ref. 3) discussed computer comparisons of agricultural features when photographed from a spacecraft using four cameras and filter systems. These comparisons could readily be data obtained from four portions of the spectrum through a microscope. A study was undertaken by Soffen and Sloan (Ref. 4) at the Jet Propulsion Laboratory to evaluate a system for detection of life by visual imaging. Various portions of the spectrum were considered and evaluated as well as some applicable instrumentation.

Billingsley and Lindsley (Ref. 5) described a process for the computer reduction of lunar photographic images to enhance and quantitatively record small color differences. Photographs taken through two different filters are scanned and recorded. Hamberg (Ref. 6) compared particle visibility and sizing using 4x macrophotography with 37x photomicrography. These results indicated that macrophotography with non-optimized equipment provided reasonable accuracy down to 16 microns, and that further refinement might allow the measurement of smaller particle sizes.

With the necessary modifications or refinement of the techniques utilizing reflected light microscopy, photography and multispectral analysis, detection of microorganisms and particulates on spacecraft and other surfaces seems likely.

The primary objective of this task is to develop new and more efficient techniques for the detection and enumeration of microbes on surfaces. Major emphasis will focus on developing techniques which require no intimate contact, that will provide direct measurements of bio-load, and will provide a permanent record and produce real-time quantitative data. If such a system can be developed, it will prove invaluable as a basic research tool, possible assay of spacecraft hardware, and sample return analyses.

Several techniques will be examined, among which will be photoacoustic spectroscopy and the system utilizing macrophotography and multispectral analyses.

The technique coupling macrophotography and multispectral analyses will attempt to convert technology which allows remote detection of biosystems on a macroscopic scale, i.e., multispectral sensing of agricultural and forest crops as well as marine populations, to allow the detection of microscopic biosystems such as microorganisms on a surface.

5.3.2 Approach

Preliminary activities involved the formulation and testing of macrophotographic specifications (films, lights, filters, exposure time and camera set-up). The results of these preliminary activities were used to develop a schematic outline of the biodetection system. The system consists of three main components - specimen, photography and image processing as shown in Figure 5-C.1.

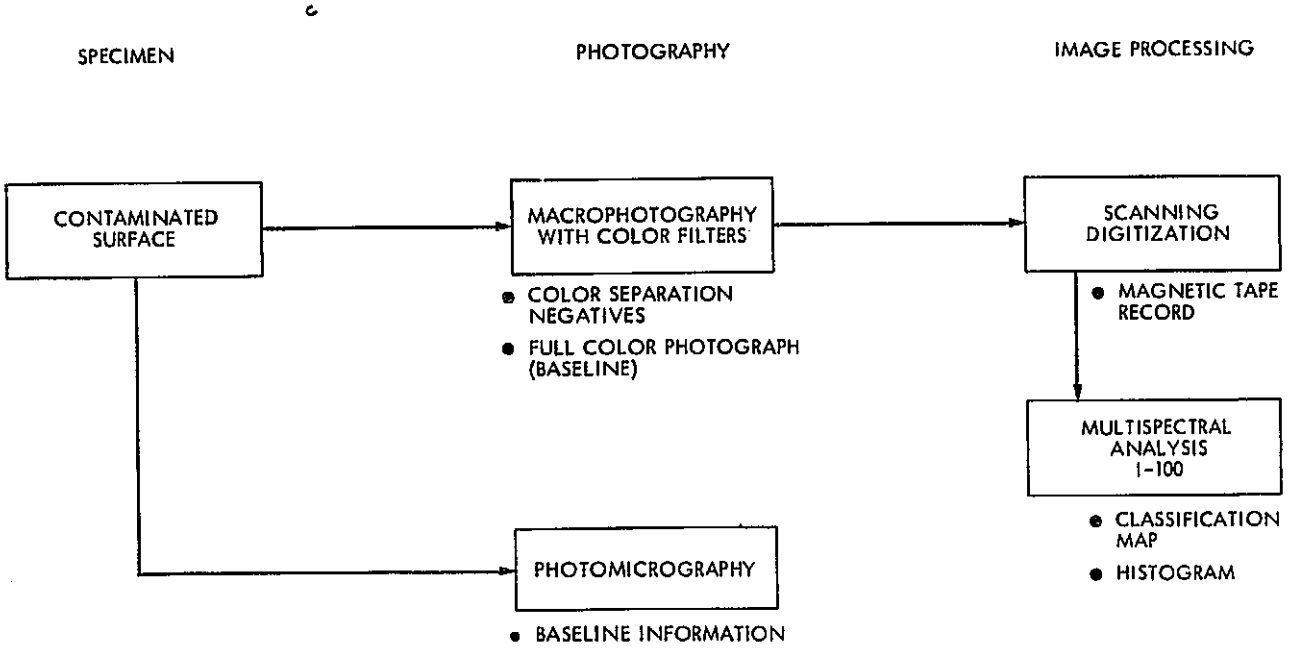

Fig. 5-C.1. Biodetection system

Further testing of the system identified several potential problem areas associated with the illumination - camera-film components. The unstable camera set-up, low-intensity light requiring long exposure times resulted in difficulty maintaining focus and image sharpness. The high-contrast film used to enhance the differences in optical densities produced by the light intensity through the different absorption filters, has a greatly reduced latitude resulting in difficulties matching exposure and processing to the film characteristics. Therefore, the preliminary testing was modified and repeated to some extent.

Two specimen, Bacillus subtilis var. niger (spore) and No. 5 vegetative were used in these tests. These specimen were deposited on several types of background slides; aluminized glass, dull aluminum, black coated glass and aluminum and polished aluminum.

The same wratten filters used in previous tests were utilized; neutral 1.9 ND (Neutral Density), blue 47B, green 55 and red 29.

In order to eliminate some of the variables and allow proper evaluation of the various components of the overall system, the camera set-up was not used. Instead, JPL's Automatic Light Microscope System (ALMS) was used to produce the images subsequently analyzed for visual spectral information.

The ALMS is an integrated system that consists of a light microscope coupled to an optical scanner which is coupled to an A to D converter that digitizes the image in the scope's field and feeds it to an IBM 1130 computer that acts as an executive system to the automated microscope and processes the data onto magnetic tape. The microscope has an epiaxial illumination system (light source enters the scope barrel and is reflected by a half-silvered mirror through the objective onto the sample) providing convenient reproducible illumination.

Each set of digital color separation images was analyzed on the G. E. Image-100 (I-100) system (Figure 5-C. 2), to determine the existence and degree of a multispectral signature associated with the specimen.

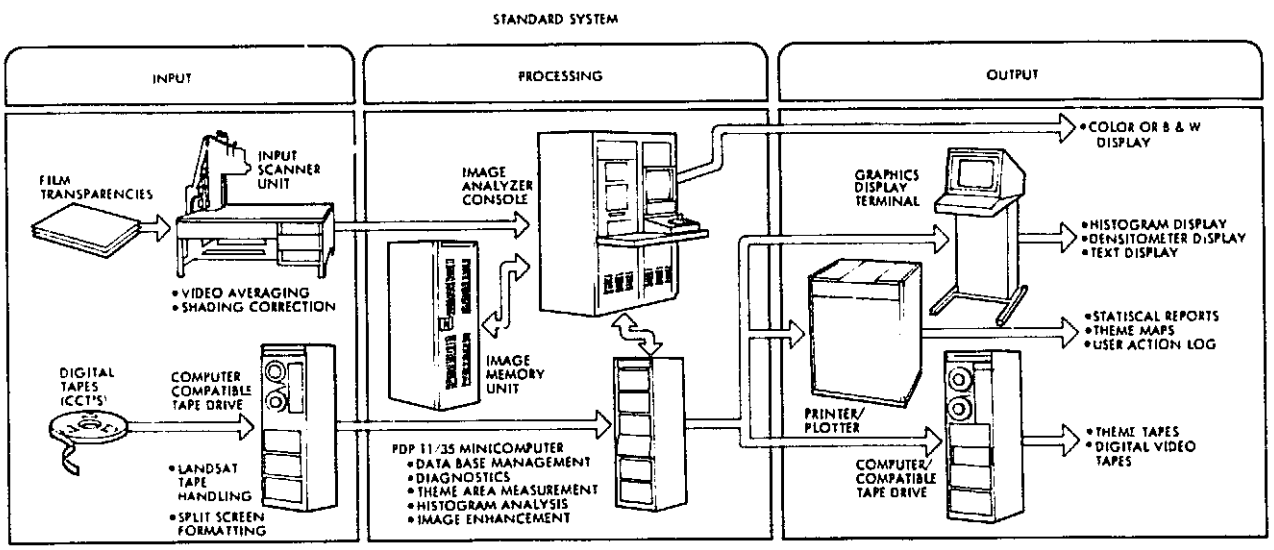

Fig. 5-C. 2. Image - 100 system configuration

5.3.3 Significant Accomplishments

During this somewhat extended feasibility period, preliminary photographic specifications, data processing, scanning and multispectral analysis were modified and retested.

An experimental test matrix that reflects adoption of the ALMS is shown in Table 4-D.1. Preliminary activities that preceded establishing the matrix included: research effort to identify the source of spectral information, familiarization with the ALMS and checking its epiaxial illumination capabilities.

5.3.3.1 Macrophotography. The camera- set-up was evaluated relative to its stableness and light source. Two proposed camera set-ups have been studied. The set-up shown in Figure 5-C.3 has been selected for fabrication.

A follow-up study is being done by the JPL Photolab relative to matching exposure and processing to the high-contrast film characteristics.

5.3.3.2 Multispectral Analyses. It has been demonstrated that Bacillus subtilis var. niger can be distinguished from other components added to a prepared slide, by spectral analyses using the G.E. Image - 100 system.

5.3.4 Future Activities

The experimental matrix will be tested and should confirm previous evidence for spectral information on images of dense microbial populations utilizing low magnification. Also, provide some information pertaining to the source of spectral information.

If the testing of the experimental matrix adequately provides the desired spectral information, then a data bank will be developed. This bank will be comprised of spectral characterization of a variety of organisms and particulates, which will be used as a basis for formulating contamination assays and other research applications.

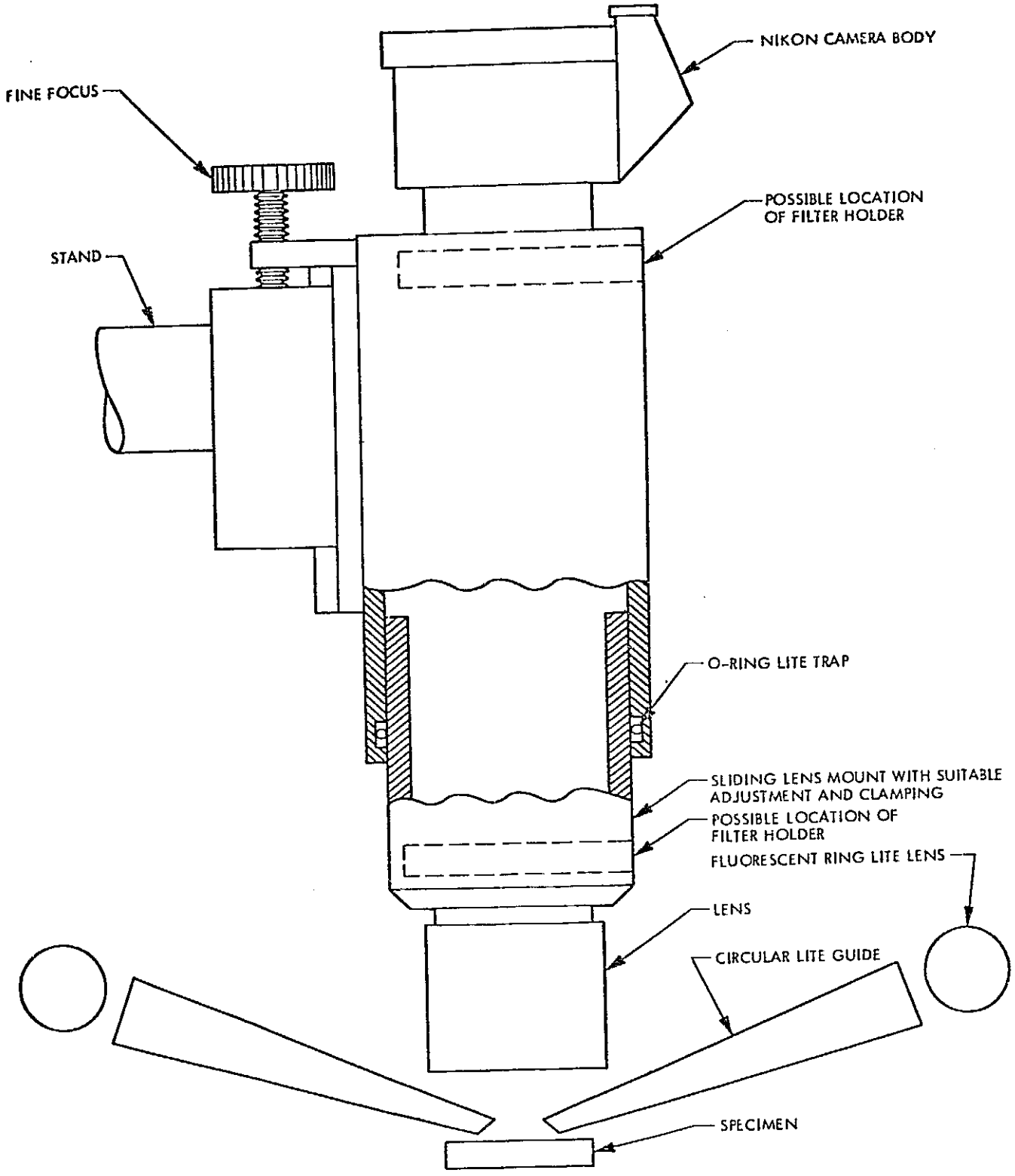

Fig. 5-C.3. Macrophotography camera setup

5.3.5 References

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SECTION VI

PLANETARY QUARANTIVE CONSIDERATIONS FOR SHUTTLE LAUNCHED SPACECRAFT (NASA NO. 193-58-63-07)

Contents	Title and Related Personnel
Para. 6.1	Planetary Quarantine Considerations for Shuttle Launched Spacecraft Cognizance: M. R. Christensen

SECTION VI

PLANETARY QUARANTINE CONSIDERATIONS FOR SHUTTLE LAUNCHED SPACECRAFT

6.1 INTRODUCTION

The objectives of this task are to assess the effect of baseline and alternative Shuttle Transportation System (STS) ground, launch, on-orbit and abort/return operations on planetary spacecraft (S/C) to determine if they could potentially impact quarantine requirements.

6.2 SIGNIFICANT ACCOMPLISHMENTS

During the report period, three major activities were completed. The first dealt with performing a particulate contamination assessment of the STS ground, launch and on-orbit operations and identifying significant contamination events. Particular attention was paid to new operations that were unique to a STS launched spacecraft.

The second activity was coupled with the contamination assessment by considering the effect various shroud options would have in attenuating identified contamination effects.

The third activity was an extension of a previous effort relating to applicability of state-of-the-art quarantine techniques and methods to the shuttle era. Although most of the techniques are usable, several deficiencies limit their value. This activity focused on identification of the technology that the quarantine program will require to assure that meaningful sampling and monitoring data can be obtained in a valid and timely manner.

6.3 RESULTS

The results of the efforts are summarized in the following sections:

6.3.1 Contamination Assessment/Shroud Effects

6.3.1.1 Shroud Options Considered. The study considered three shroud options, these being: 1) No shroud — This option, which is desirable due to

the lack of weight penalties, assumed that the STS payload bay was enclosed with a flexible liner, 2) Reusable shroud - This option, which is unlikely but still being considered, assumed that the reusable shroud was an inherent component of the orbiter structure, and 3) Disposable shroud - This option assumed that the disposable shroud would be similar to the Mariner 1971 shroud, i.e., clam-shell structure opening along a vertical split line.

6.3.1.2 Study Definitions. For the purposes of this study, a contamination event was defined as any event where a high probability existed for the generation of particulate (dust, water, fibers, skin, etc.) and non-particulate (films, etc.) matter, both volatile and nonvolatile in nature. These events were identified because of the relationship between particulate and microbial contamination, in which greater numbers of particulates generally equate to higher microbial levels.

In a similar manner, quarantine events were identified in qualitative terms, for example, identifying situations (using past experience) where the nature and degree of contamination might necessitate a reprocessing (reclean, decontamination or sterilization) of the spacecraft in order to meet Planetary Quarantine (PQ) requirements. These requirements are developed and imposed as NASA policy, in conjunction with the National Academy of Science's Space Science Board. The type of requirements imposed on a Project are mission dependent.

6.3.1.3 Study Assumptions. The contamination assessment was conducted using the following assumptions:

1) An MJO-type mission was used as the study model.

2) The spacecraft was installed vertically on the pad with the interim upper stage (IUS) already in the payload (P/L) bay.

3) A reusable shroud would employ gasket-type seals at all closure points, including the adapter and IUS.

4) Contamination and quarantine event identification were based on Viking type PQ requirements.

5) A liner of non-shed material would be installed in the P/L bay to minimize contamination from orbiter structural and support elements.

6) The P/L bay would be no better than Class 5, 000 environment prior to launch.

7) The abort and reentry mode were not considered to any detail, since any shroud option other than that of the pressurized disposable shroud would subject the spacecraft to an influx of 50 micron or greater particles and minimal humidity control.

6.3.1.4 Approach. The approach was to examine the shroud options in relation to the typical operations that would occur during each mission phase in an effort to identify the contaminating events. It was understood that this was a first-cut assessment performed in lieu of exacting detail regarding each operation. The type of contamination, the probable cause of the contamination, and the suggested method of control were noted along with any salient comments regarding the particular operation.

6.3.1.5 Conclusions. The study conclusions are qualitative in nature and are reported in terms of the number and type of contaminating events. It was assumed, however, that each identified event could potentially have a deleterious effect upon the spacecraft due to either an impact on contamination sensitive hardware or by exceeding the PQ imposed microbial burden requirement.

Quantitation of the level of contaminants associated with each event would be dependent upon obtaining and understanding data for several variables, for example, the duration of the operation, the number of personnel involved, the level of environmental control and other important factors.

Table 6-1, pages 6-8 through 6-11, indicate that of the 24 identified operations that occur up to the time of IUS ignition, 22 have some effect on a no-shroud system; 20 have an effect on a reusable shroud system; and only 8 have an effect on a disposable shroud system.

In comparing the no-shroud and reusable shroud system, it should be noted that although the number of contaminating events are similar, the potential for, and the magnitude of, contamination remains greater for a no-shroud system.

For the abort-reentry mode, a review of present plans indicates that both the no-shroud and reusable shroud options would subject the spacecraft to an influx of 50 micron or greater particles and minimal humidity control. Such a result would necessitate total reprocessing of the spacecraft for PQ and contamination control (C^2) requirements. Prevention of this problem with a disposable shroud would be dependent upon the ability to maintain the shroud interior above ambient and the sustaining of a GN_2 purge.

The results further indicate that if the no-shroud or reusable shroud options are employed, the nature of the prelaunch operations are such that pre-pad PQ sampling could be invalidated. Invalidation could necessitate resampling on the pad, an operation that requires spacecraft access for 6-8 hours and an additional 40-72 hours for the samples to be processed, enumerated, and presented for NASA consideration.

Two additional problems deserving consideration were revealed as a result of the assessment. The first deals with the proposed Class 5,000 clean room on the pad, the Payload Changeout Room (PCR). Drawing on our past clean room experience, it would be our contention at this time, that PCR appears inadequate for PQ constrained spacecraft operations. Until more operational details become available, it is suggested that personnel numbers and equipment within the room would have to remain limited, stringent cleaning operations would have to be implemented, and a continuous air quality monitoring capability should be provided. The second problem deals with on-orbit operations. Our preliminary assessment indicates that, depending upon the nature and magnitude of the off-gas, out-gas and molecular cloud formations, a 3-6 hour on-orbit dispersion interval may be required prior to deployment of the spacecraft from the P/L bay. Failure to implement such a procedure (for the no-shroud and reusable shroud options) could result in severe degradation in spacecraft cleanliness and performance. It is unknown, at this time, what effect orbital

maneuvering would have in shortening the dispersion interval. It should be noted, however, that fairly vigorous on-orbit contamination requirements are being requested by the STS Program Contamination Requirements Definition Group.* At this stage of the program, however, it is unknown whether the operational STS can achieve them.

As a result of the preliminary assessment, it is felt that the no-shroud and reusable shroud options result in high contamination risks for the spacecraft (particulate and microbial). Through use of the disposable shroud options, the P/L community could, on a mission-by-mission basis, achieve cleanliness standards greater than those presently proposed by the STS, at a reasonable cost and with minimal impact on STS operations and facilities.

6.3.2 Quarantine Monitoring/Technology Needs

This effort evaluated the application of the state-of-the-art quarantine technique used to obtain sampling and monitoring data. These techniques were assessed in light of proposed STS operations and constraints. The results of the evaluation indicated that the Viking-type techniques were indeed usable; however, several deficiencies limited their value.

The type of technology required to remedy the problems was identified. In brief, they are:

1) Development of a technique to sample large STS surface areas, specifically the P/L bay. Also a technique that could be applied to the P/L bay liner which is a fabric-type material.

2) Development of statistically sound extrapolation techniques. Present techniques of extrapolating 4 square inches (in^2) sample data to larger surfaces may be woefully inadequate when applied to a 60 by 15 foot cylinder.

3) Development of rapid-assay techniques. This will be particularly important as the S/C approaches entry into the 160 hours STS turnaround timeline or for S/C malfunction problems which will necessitate resampling of the S/C surfaces.

*"Payload contamination control requirements for SB induced environment," MSFC, July 1975.

4) Development of a technique to obtain large surface area, short duration samples. Somewhat related to item 1) but with the element of urgency included. This may be necessary to limit technician exposure time to potential radiation hazards.

5) Development of a technique to obtain on-orbit microbial samples. Recontamination estimates, for quarantine purposes, will require knowledge of on-orbit P/L contamination. Although particulate contamination data will be available, microbial data will not. Such data would also be required to support a no-shroud/liner only decision.

6) Development of a predictive program to determine probable contamination based on specific operations. Obtaining real-time data on quarantine flights may be cost prohibitive, particularly on-orbit. Data obtained from early orbital flight tests (OFT's) could be used in a computer program developed at JPL to develop predictive processes that are able to bound contamination levels, given the knowledge of the type and extent of operation.

7) Development of rapid cleaning techniques that can be applied to large surface areas. Efficiency of removal factors for an acceptable cleaning technique must be high for both particulates and viables. The technique should be such that typical hardware compatibility problems can be by-passed (e.g. electrostatics). Such a technique would be extremely valuable in reducing microbial loads prior to sterilization/decontamination, or to remove large particulates ingested during an STS reentry mode.

8) Development of a technique to monitor particulate and microbial contamination within the shroud environment. Viking experience indicated that such a technique would be a valuable quarantine Project tool in assessing problems associated with in-shroud contamination distribution and/or air-conditioning/purge failure.

6.4 FUTURE ACTIVITIES

Review of STS operational concepts will continue per plan. Efforts will be intensified to develop plans to define how and when specific contamination data may be obtained, for example, the contamination levels of the operational payload changeout room (PCR), or STS P/C bay assays.

Emphasis will be placed on the results of the task to define technology and requirements to meet quarantine program needs for effective shuttle interfacing. Technology readiness dates will be determined.

Table 6-1. Contamination assessment MJO type spacecraft

Shroud Options (x) Potential S/C Contaminating Event			Mission Phase	Operation	Probable Cause of Contamination	Primary Type of Contamination	Suggested Method of Control	Type of Event	Comment
Disposable Shroud	Shuttle Shroud Reusable	No Shroud							
X	X	X	Pre-launch	1. Conduct PQ Biol. Monitoring Ops	Particulate Shedding from Personnel, Atmospheric Contaminants, Sampling and Cleaning Media	Particulate, Biological, Water Vapor, Isopropyl	Use of Clean Room Garments (CRG), Clean Room Air Stds. (CRAS), Handling and Cleaning Procedures	PQ	PQ Significant Missions only. 6-8 hours.
X	X	X		2. Prepare for Trans. to Pad, Encapsulate				PQ and C^2	
				A. Cannister	Crane, Particulate Shedding from Personnel, Atmos. Contaminants	Biological, Particulate	Crane Cover, Line Umbrella, CRG, CRAS, Positive Purge, Can Clean	PQ and C^2	
				B. Flexible Cover	As above with Addition of Plastic Mat'l Shedding	Biological, Particulate	As above with Addition, Non-shed Plastic Mat'l	PQ and C^2	
				C. Disposable Shroud	As above.	Biological, Particulate	As above, with Shroud Cleaning		
X	X	X		3. Transfer to Pad	Particulate Migration and Redistribution from S/C Structure and Cover	Particulate	Maintenance of Clean S/C prior to Encapsulation will Minimize, Set and Achieve Cleanliness STD for Interior or Can, Cover or Shroud	C^2	
X	X	X		4. Move S/C to Pad Apron and position	Particle Migration and redistribution from S/C Structure and Cover	Particulate	As above	C^2	
	X	X		5. Lift S/C into Payload Changeout Room (PCR)	As above	Particulate	As above	C^2	
	X	X		6. Remove S/C Cover	Particulate Shedding for Personnel, Flexing of Cover, Crane Ops, Atmospheric Contaminants	Biological and Particulates	CRAS, CRG, Clean Ext of Cover prior to Removal, Crane Cover, Line Umbrella	PQ and	*PCR must run for extended period of time following closure around Shuttle to permit cleanup and stabilization. If PCR is retracted for period of time, flooring and surfaces should be cleaned.

NOTE: From this point on, Events 5-14 and 17-22 only affect this option in terms of potential recontamination of the S/C at shroud separation.

*Potential Significant Time Impact Not Previously Considered.

Table 6-1. Contamination assessment MJO type spacecraft (Contd)

Shroud Options (x) Potential S/C Contaminating Event			Mission Phase	Operation	Probable Cause of Contamination	Primary Type of Contamination	Suggested Method of Control	Type of Event	Comment
Disposable Shroud	Shuttle Shroud Reusable	No Shroud							
	X	X		7. Open P/L Bay Doors, Extend RR Ant. and Flt. Manipulator Arm	Particulate Cloud Dispersion for opening Shuttle P/L Doors and Use of GSE. Particles Generated for Extension of Manipulator and Flexing of Bay Liner	Biological and Particulate	Clean GSE and P/L Doors prior to opening, retain S/C in Covered Condition until Completion of this Operation, Cover Manipulator Actuators	PQ and C^2	*Exposure of Shuttle surfaces to Cape Environment could necessitate extensive cleaning of external areas which lie within PCR
	X	X		8. Open Shuttle Shroud (Reusable)	Particulate Cloud Dispersion for opening Shroud Doors and Use of GSE, Flexing of Seals	Biological and Particulate	Clean Exterior of Shroud Doors just Prior to Opening. Clean GSE, Clean Seals	PQ and C^2	Assumes P/L Bay and Interior of Shroud have been cleaned to some acceptable cleanliness standard and maintained in that condition, including bay liner
	X	X		9. Lower TUG strongback, Attach S/C	Particulate Generation for Strongback Movement, Particulate Shedding for Personnel, Insufficient Clean Room Air Classification	Biological and Particulate, Potential Film Formation	Clean and Cover Strongback Pulleys and Moving Systems, Localized Purge of S/C and Critical Elements, Minimization of Personnel in PCR	PQ and C^2	Assumes Strongback in PCR for Vertical S/C installation
	X	X		10. Extend S/C into P/L Bay, Mate to IUS, Verification Tests	Particulate Generation for Movement of Strongback Components, Personnel Concentration in Limited Work Area, Insufficient Clean Room Air Classification	Biological and Particulate, Potential Film Formation	As above, with Addition of Increasing P/L Bay Purge Flow Prior to S/C Installation and Maintenance at High Rate per Comment	PQ and C^2	PCR Environment will deteriorate rapidly with increased activity. Suggest diversion of HEPA filtered air in pattern and site to reduce localized particulate for settling on S/C
	X	X	Pre-launch	11. Disconnect, Extract Strongback for P/L Bay	Particulate Generation for Movement of Strongback Components	Particulate and Potential Film	See No. 9	PQ and C^2	
	X	X		12. Close Shuttle Shroud Doors (Reusable)	Particulate Cloud Dispersion for Closing Shroud Doors, Flexing and Compression of Seals, Flexing of P/L Bay liner. Use of GSE.	Particulate	See No. 8	PQ and C^2	At this stage, all additional Pad operations in the P/L vicinity have no direct affect on the S/C other than as potential recontamination events

*Potential Significant Time Impact Not Previously Considered.

Table 6-1. Contamination assessment MJO type spacecraft (Contd)

Shroud Options (x) Potential S/C Contaminating Event			Mission Phase	Operation	Probable Cause of Contamination	Primary Type of Contamination	Suggested Method of Control	Type of Event	Comment
Disposable Shroud	Shuttle Shroud Reusable	No Shroud							
		X		13. Retract RR Ant. Flt Manipulator Arm and Close P/L Door	Per No. 7	Biological and Particulate	See No. 7	PQ and C^2	P/L bay closure could create turbulence which could potentially dislodge particulates for IUS
				14. Closeout Ops/ Clear Pad	No Significant Affect	No Significant Affect	No Significant Affect	No Significant Affect	*Nature of Ops 2-13 are such that initial PQ monitoring data may be invalidated. Resampling could require 6-8 hrs. just prior to close up
X	X	X	Launch and Ascent	15. Liftoff/SRB Burn/ SSME Burn	Particulate Migration and Redistribution Due to Acoustics and Liftoff; Influx of Ambient Atmosphere just Prior to liftoff, P/L Components begin outgassing	Biological and Particulates, Potential Films from Outgassing	Minimization through Maintenance of GN_2 Purge from Orbiter	PQ and C^2	It is likely that outgas problems are minimized in the design, Mgf., test, and bake-out of hdw. Potential contamination for proposed liner (i. e., billowing or filter integrity) unknown at this time.
X	X	X	Orbit Insertion	16. OMS Burn and Orbit Insertion	Exterior: Combustion Residues for Propulsive System, Particle Migration along Body of Vehicle, Outgas and Offgas Cloud Formations. Interior: Particle Migration and Redistribution due to Acoustics and Vibration, Outgas and Offgas Cloud and Molecular Formations	Particulates Films Volatiles	Exterior: No Fix Interior: Maintenance of GN_2 Purge for Orbiter	C^2	Assumes liner and supporting filter integrity maintained.
		X	On-Orbit	17. Open P/L Bay Doo. s, Cruise, Adjust Orbit	Particulate Entry for Vehicle Ext, Particle Dislodgement for Movement of P/L Doors, Dispersion of Clouds, Venting for Orbiter, RCS Contaminants	Particulates Films Volatiles	Allow Normal Atmospheric Dispersion Process, Inhibit Venting and Dumps During Remaining On-Orbit Phase, Inhibit RCS Firings to Minimum for Remaining Operations	C^2	*Dispersion of particulates in size dependent, larger particle could take 4-6 hours. With an unshrouded S/C some orbital maneuvering should occur prior to P/L Bay Door opening

*Potential Significant Time Impact Not Previously Considered.

Table 6-1. Contamination assessment MJO type spacecraft (Contd)

Shroud Options (x) Potential S/C Contaminating Event			Mission Phase	Operation	Probable Cause of Contamination	Primary Type of Contamination	Suggested Method of Control	Type of Event	Comment
Disposable Shroud	Shuttle Shroud Reusable	No Shroud							
	X	X		18. Deploy Manipulator, Open Reusable Shroud or Remove RTG Cooling Jackets	Particulate generation for Release of Manipulator, Use of Actuators and End Effector, Opening of any Shroud Latch Mechanisms, Opening of Reusable Shroud, Particulate Redistribution	Particulates Potential Films	Manipulator Hdw must be Thoroughly Cleaned prior to Launch, Use of Flexible Non-shed Sleeves on Actuators	C^2	Attention should be paid to where particles generated for this operation might lodge since S/C recontamination potential could be significant
	X	X	On Orbit	19. Connect Manipulator to IUS	Particulates for Actuators and Effector	Particulates Potential Films	Per Item No. 18	C^2	Per Item No. 18
	X	X		20. Release Latches, Disconnect Umbilical	Particulate Generation for Release of Tension Latches	Particulates	Some Shielding for Proposed Liner, Prelaunch Cleaning of Latches	C^2	
	X	X		21. Deploy and Release P/L	Particulates Generated for Manipulator, S/C brought above P/L Bay Plane, Possible Contact with any Remaining Particulate and Molecular Clouds, Possible RCS Contaminants	Particulates Potential Films Volatiles	Automated Optics and Sensor Covers, Inhibit RCS Correction to Bare Minimum, Delay Deployment Until Dispersion Processes Complete	C^2	
	X	X		22. Orbiter Fly Around and Visual Inspection	Potential RCS and Venting Contaminants	Particulates Volatiles	Orientation of S/C or Orbiter to Prevent Line-of-Sight Contamination Potential	C^2	
X	X	X		23. Activate Centaur Ignition	Particulate Migration and Redistribution	Particulate	No Fix	C^2	
X				24. Jettison Shroud	Particulate Generation for Zip Line Detonation and Shroud Separation. Recontamination for Shroud to S/C	Biological and Particulates	No Fix. Past Experience has not indicated problem	PQ and C^2	

*Potential Significant Time Impact Not Previously Considered

SECTION VII

QUARANTINE CONSIDERATIONS FOR SAMPLE RETURN MISSIONS (NASA NO. 193-58-64-01)

Contents	Title and Related Personnel
Para. 7.1	Quarantine Consideration for Sample Return Missions

Cognizance: M. R. Christensen

SECTION VII

QUARANTINE CONSIDERATIONS FOR SAMPLE RETURN MISSIONS

7.1 INTRODUCTION

The objective of this task is to identify the quarantine aspects of sample return missions and describe them in terms of mission events and technology needs. Several approaches can be utilized in meeting these objectives. For this reporting period the task objectives were directed to identifying the major quarantine planning functions that would be required to respond to a Mars Surface Sample Return (MSSR) mission.

7.2 PURPOSE

During this reporting period the purpose of the task was to assemble a study team to preliminarily address MSSR quarantine issues which by their nature:

1) are critical to front-end planning of a sample return mission and/or
2) contain elements that are potentially controversial either scientifically or politically and/or
3) could severely impact spacecraft design, operations or facilities.

7.3 APPROACH

The following approach was used:

1) a multi-organizational team was formed to conduct the task,
2) a "strawman" mission based on a 1984 launch was established,
3) the primary quarantine problems associated with planning and implementing an MSSR mission were identified,

4) top level mission "critical" tasks were extracted from this list on the basis of the priorities discussed in the purpose section of this report, and

5) the tasks were assigned to the appropriate task members.

It was the responsibility of the task members to perform a "problem definition" study for their particular quarantine problem. It was never the objective of the individual tasks, however, to resolve the problem; just to carry it to the point where it could be understood at the level necessary to perform logical planning functions. The task leaders were free to use any assumptions they felt were necessary to define the problem, as long as they were justifiable. The selected tasks, task leaders, and "problem definition goals" are presented in Table 7-1.

The expected outcome for the study was the preparation of a document that suggests, within the context of a "reference" mission, the preliminary assessment, planning, and implementation required to resolve several critical quarantine problems.

7.4 SIGNIFICANT ACCOMPLISHMENTS

The team has been assembled, tasks selected, assigned and carried to completion. The mid-term results were discussed in an ad-hoc meeting at COSPAR in Philadelphia, Pa., in Jung of 1976. An in-house (JPL) critique was conducted in July. In August the study was expanded so each task would include:

1) a strong "stand alone" summary indicating, task description, justification, and cost data,

2) a planning structure carried to the sub-task level, including subtask cost estimates and

3) identification of the primary drivers, specifically cost or technology.

C-2

Table 7-1. MSSR Quarantine Planning Study Tasks, Participants, and Goals

Task	Participant	Goal (Problem Definition)
1. Definition of Interim Quarantine Reqs	Exotech Inc. Mr. Robert Wolfson	Prelim Set of Interim Reqs (Qualitative in Nature)
2. Effect of Viking Results on Outbound Quarantine Reqs	Exotech Inc. Mr. Pericles Stabekis	Prelim Impact Assessment
3. Quarantine Protocol	Biospherics Inc. Dr. Gilbert Levin	Prelim Protocol Sample Estimate for Quarantine Purposes
4. Sample Canister-Containment and Verification	JPL Mr. Mike Wardle	Technology Assessment - Prelim Impact Assessment
5. Science vs Quarantine Trade-Offs	JPL Mr. Marvin Christensen	State of Knowledge Relative to Competing Science and Quarantine Reqs
6. Planetary Receiving Lab Study	Ames Dr. Donald DeVincenzi	Preliminary Facility Reqs. - Available Facilities
7. Biosafety and Containment - Management Considerations	Colorado State University Dr. John Bagby	Potential Participants, Past Problems, Approach to Achieve Effective Management Structure

7.5 RESULTS

The results of this task will be presented in detail in a review to be held November 17, 1976. A final report will be issued as a formal JPL document in February, 1977.

7.6 FUTURE ACTIVITIES

Suggested follow-on activities will be developed for each task area. These will be presented to our NASA sponsor for his consideration during February/March of 1977.

SECTION VIII

CONTAINMENT TECHNOLOGY FOR SAMPLE RETURN MISSIONS (NASA NO. 195-58-64-02)

Contents

Title and Related Personnel

Cognizance: R. Koukol

Associate Personnel: M. Wardle

SECTION VIII

CONTAINMENT TECHNOLOGY FOR SAMPLE RETURN MISSIONS

8.1 REMOTE HANDLING TECHNOLOGY FOR EXTRATERRESTRIAL HAZARDOUS MATERIALS

8.1.1 Introduction

A successful Viking mission to Mars may find viable life forms, traces of past life, or the chemical precursors to life. A natural follow-on mission would be to return to Mars (or any other planet of biological interest), obtain a soil sample and return it to earth for analysis. To preserve the integrity of this sample material for science objectives and to insure that the terrestrial biosphere is not compromised, some type of containment system must be used. Whenever a researcher is isolated from the sample material by a containment system, some restraints are put on his ability to manipulate the sample. The most stringent containment systems to date used for quarantine purposes have been the Lunar Receiving Laboratory class III gastight gloveboxes.

Another possible solution to the problems of handling sample material and equipment in a containment system is through the use of teleoperator or remote manipulator control systems. A teleoperator may be defined as "a general purpose, dexterous, cybernetic machine." (Ref. 5) The "tele" in the word describes the ability of this type of machine to project or extend a man's dexterity across great distances or solid barriers. A remote manipulator is a machine which possesses the capability to perform tasks which normally require the dexterity of a human hand and arm. (Ref. 5) The distinction between the two is so fine that for purposes of this report the two terms will be used synonymously.

This objective of this study was to assess the state-of-the-art of remote manipulator or teleoperator systems as it pertained to their suitability and adaptability for handling extraterrestrial biohazardous material.

This report will be presented in three parts: background and rationale; review of remote manipulator technology; and assessment of the technology using criteria of pertinence to quarantine.

8.1.2 Approach

8.1.2.1 Background and Rationale. In the study of pathogenic and potentially pathogenic biological material, a consideration must be made of the infectivity and virulence of the biotic material and the effectiveness and reliability of any barrier system between or surrounding the researcher and the experimental materials. Methodologies which are very successful in maintaining experimental isolation of the researcher from material of low infectivity or poor stability may be totally inadequate for highly biohazardous material. This is most readily documented in the experience of the Biological Warfare Laboratory (B. W. L.) at Fort Detrick, Maryland. In the early days of biological research, only the most rudimentary precautions were undertaken to maintain the isolation of the sample for the protection of the researcher. As a result, many laboratory induced infections occurred including some to individuals who never set foot in the laboratory. These individuals were, however, associated with some aspect of the laboratory (laundry workers, spouses, working in adjacent area, etc.) (Ref. 3). In an attempt to reduce possible infections, the B. W. L. followed four approaches (Ref. 1):

1) immunization of personnel,

2) testing to determine the presence of hazardous organisms and decontaminating to remove them,

3) developing safe laboratory methods to reduce hazardous conditions, and

4) developing biobarriers to contain biohazardous material.

However, when the biological material is virtually unknown such as life from another planet or recombinant genetic material, not all of these approaches are practical; e.g., immunization.

The purpose of a sample return investigation is to determine the presence and nature of biological material. Thus the approach to the safe manipulation of a return sample is to develop handling systems in conjunction with biobarriers to contain potentially biohazardous material.

The maximum containment system deviced by the B. W. L. is the class III gas tight cabinet containment system. The class III gastight cabinet system is totally enclosed with filtered air supplied thr ough the cabinet and out through the exhaust system. The class III cabinet may be modified for special functions. In addition to use as containment systems, cabinets have been used in series with backmounted incubators, bottom mounted freezers, refrigerators, centrifuges, sonicating devices, and built-in microscopes (Ref. 6, 7).

The class III cabinet was used to maintain the lunar samples returned for analysis during the Apollo program (Ref. 9, 10). The adequacy of these cabinets in the lunar program has been questioned (Ref. 2, 11, 12); hence their appropriateness for handling the more potentially biohazardous return material represented by, for example, a Mars sample return mission is dubious. Complaints against these cabinets include biobarrier failure and difficulty in operating through the gloves of the system (Ref. 2). The B. W. L. has documented instances of class III cabinet failure leading to laboratory infections (Ref. 3).

There are a number of locations in the gloves boxes where leaks most commonly occur; the air lock, dunk tank, and pass box used in passing material through the barrier as well as the rubber gloves.

Hazards due to the air lock can be minimized by reducing traffic through the interfaces of the biobarrier. Minimization of material traffic can be accomplished in part by having well planned operations and in part by having a continuous line of biological safety cabinets. This allows material to be moved from one work station to another without having to be removed from behind the biobarrier.

The least reliable portion of the total biobarrier system are the gloves or protective suits. Rubber gloves presently used in glove box systems have a normal service life in excess of one year; however, they are subject to many mechanical failures. They can be punctured, burned, torn by sharp objects, or cut by broken glassware. A very common occurrence in animal work is researcher self-innoculation by hypodermic needle.

Replacement of a glove or suit being used for handling extraterrestrial material or genetic recombinancy studies may cause a potentially hazardous situation. Thus if use of this weak link in the biobarrier system can be avoided or minimized, it should be. Use of a remote manipulator or teleoperator system would allow for the use of an absolute biobarrier system with the ability to maneuver and manipulate samples and material freely.

A containment system analogous to this class III system has used a remote manipulator system in raising germ free animals (Ref. 8). A feasibility study performed by the B. W. L. in 1960 (Ref. 1) found that using a sealed containment system (again analogous to the class III gastight system) with a remote manipulator was feasible for biological work. This study concluded "The system provides maximum biological safety, will be easy to operate, and the output per worker will be considerably higher than that in laboratories equipped with class III safety cabinets with gloves."

8.1.3 Accomplishments

8.1.3.1 Remote Manipulator Technology. While man has been fascinated by robotics for many years, teleoperator or remote manipulation technology is only about 20 years old. This technology was derived by necessity for remote handling equipment. As shown in Figure 8-1 remote manipulators are predominantly found in applications where direct human contact is not possible (radiation laboratories and undersea operations). The term "remote" refers to any location out of the reach of the human operator.

Manipulators are machines which perform tasks which normally require the dexterity of a human arm and hand. They are designed to extend, supplement or replace man in regard to material handling or using tools.

Manipulators are mechanical systems composed of a number of rigid bodies (links) joined by single-degree-of-freedom rotary or prismatic (linear) joints. These links form an open loop kinematic chain. The initial link is connected to a fixed base and the final link is connected to a free terminal device. The links are powered such that they move relative to one another. The final outcome is that of a controlled multi-motion system. This system

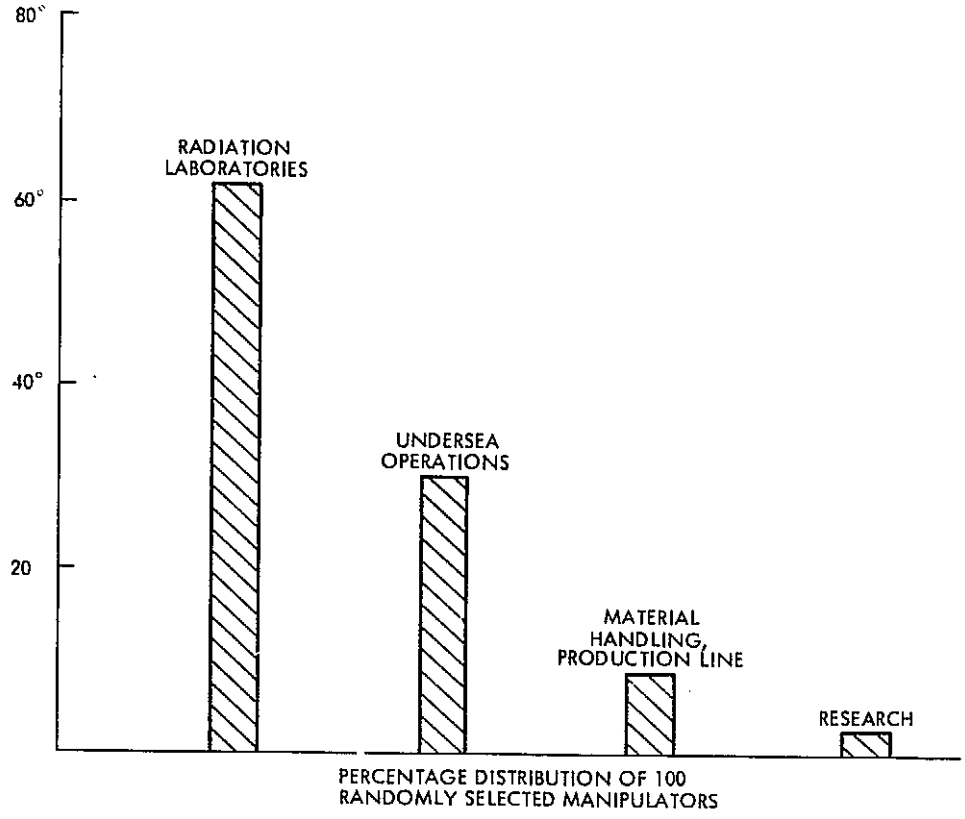


Fig. 8-1. Manipulator applications

then has "n" degrees of freedom (defined by the number of "links" in the system) to orient and position the terminal device to perform various tasks. Seven degrees of freedom (three for translation (linear), three for rotation (rotary) and one for the grasping motion) is the minimum for practical use of a general purpose manipulator as depicted in Figure 8-2. Additional degrees-of-freedom will enhance the use of the manipulator within the work space and will allow for more sophisticated handling of objects of various shapes. However, an increase in the number of degrees-of-freedom also increases the necessary sophistication of controls.

It is important to define the task operations of manipulator systems as they are paramount in defining the design of the manipulation system.

An operation characterization which may be useful is as follows (Ref. 5):

Grasping:	Finding an object fixed in space
Holding:	Maintaining an object infixed in space

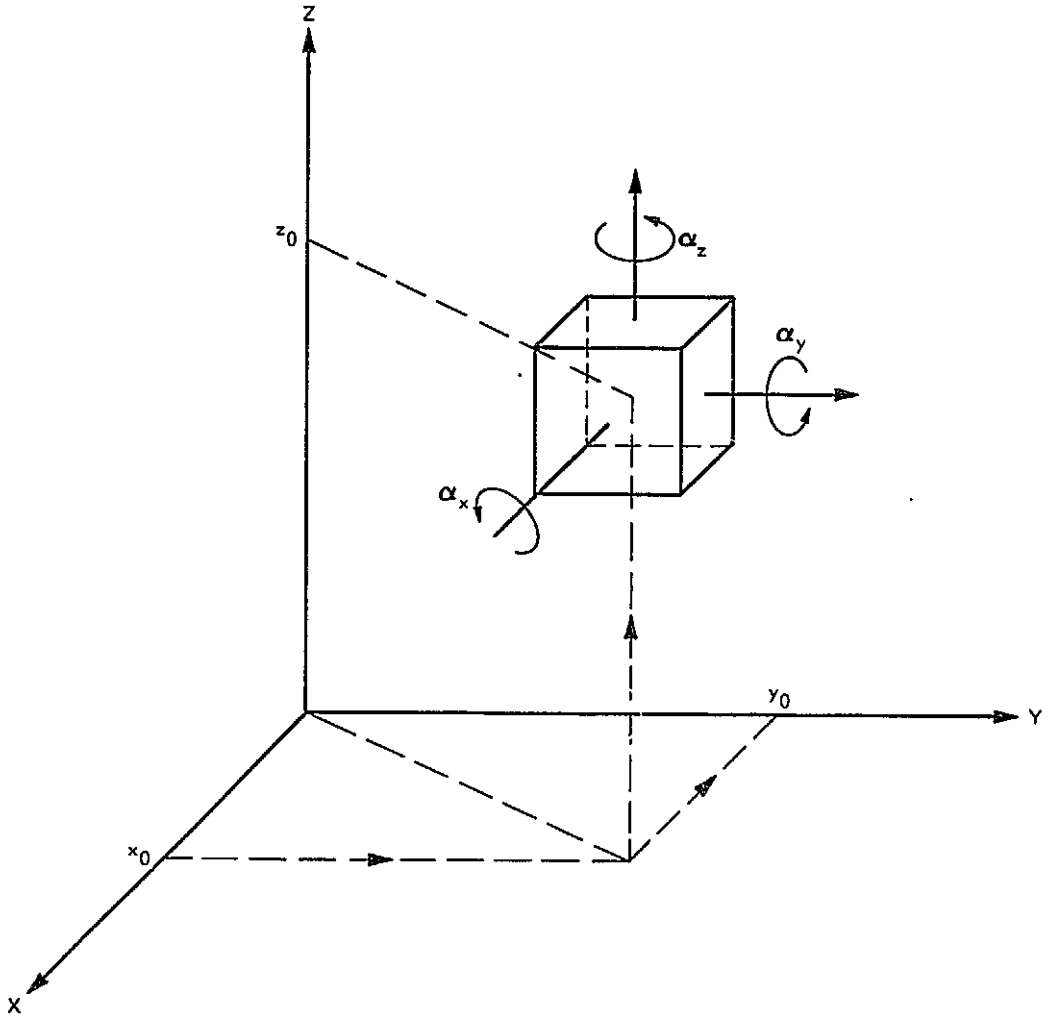


Fig. 8-2. General motions for handling objects

Moving:	Transferring an object from one point to another
Orienting:	Aligning of an object
Guiding:	Following a given path
Forcing:	Applying forces and torques
Sensing:	Forces, torques, shapes

Manipulators can be classified according to their kinematic configuration, control systems, power means (actuators), work space and work load characteristics.

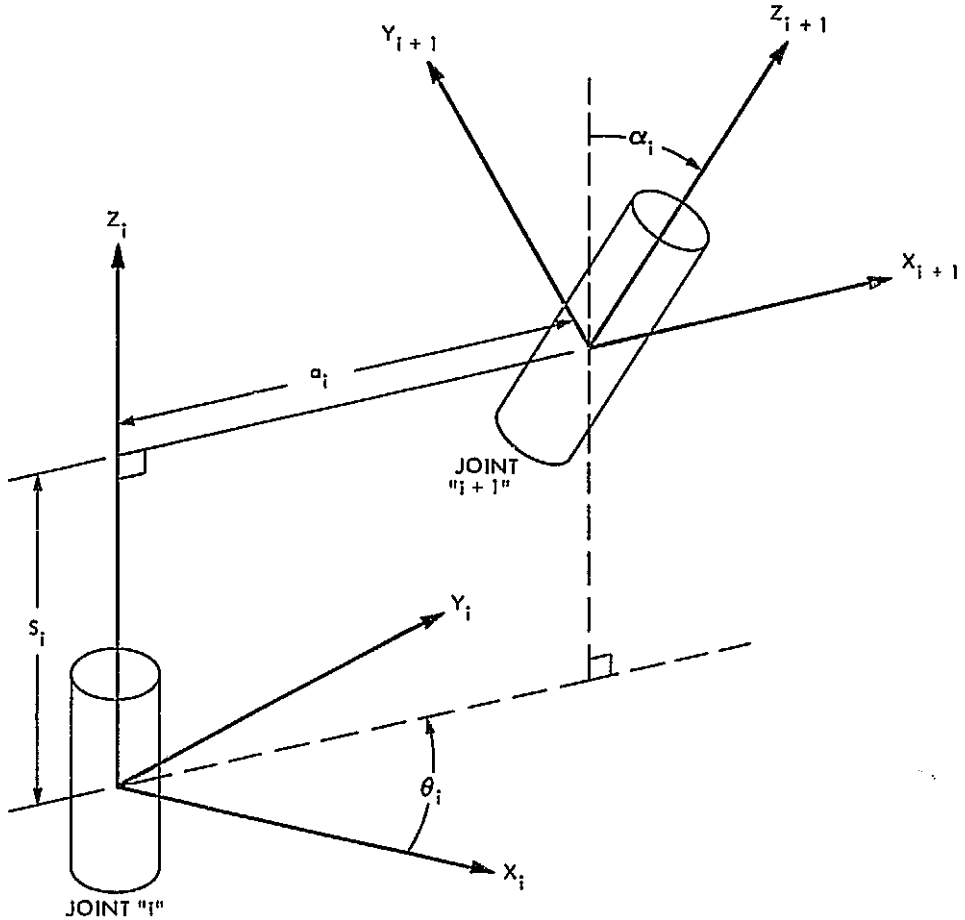
The kinematic configuration of a manipulator is related to four geometric parameters for each joint as shown in Figure 8-3. For a linear joint "i", s_i is the variable (controllable) parameter and the remaining three parameters (θ, a_i, α_i) are constants, while for a rotary joint "i", θ_i is the variable (controllable) parameter and the other three parameters (s_i, a_i, α_i) are constants. It is noted that a_i and α_i are constants for both types of joints. The subscript "i" refers to the index number of the joints - joint indexing starts at the base joint (Ref. 4).

In general, control systems can be divided into two separate classes, manual and automated. Manual control manipulator systems are completely controlled by a human operator. In this type of controller for every reaction of the manipulator there must be an initiating action on the part of the operator.

The automated or programmable control system is controlled by a computer or other programmable device. It is not necessary for a human operator to perform step-by-step detail.

The systems with a human controller who is an active or real time element in the control loop can be divided according to the method the operator uses to communicate control commands. These systems include master-slave systems, push-button control consoles and joystick-type control command arrangements. In human controlled systems, it is possible to distinguish between position control, rate control and force control techniques.

Position control refers to the face that the controlled element follows the position of the controlling element. Thus the control command is position. Rate control means that the velocity or rate of the controlled element is a function of the position of the controlling element. Thus the control command is velocity. Force-reflecting control means that forces acting on the manipulator are reflected back to the controller such that the input load will be



a_i = LENGTH OF COMMON NORMAL BETWEEN Z_i AND $Z_i + 1$

α_i = ANGLE BETWEEN Z_i AND $Z_i + 1$ POSITIVE IN THE RIGHT HAND SENSE ABOUT COMMON NORMAL

} DISPLACEMENT AND ROTATION MEASURES ORTHOGONAL TO THE AXIS OF MOTION OF BODY "i" (THESE ARE CONSTANTS)

θ_i = JOINT ANGLE, POSITIVE IN THE RIGHT HAND SENSE ABOUT Z_i

s_i = VALUE OF Z_i AT WHICH THE COMMON NORMAL INTERSECTS Z_i

} DISPLACEMENT AND ROTATION MEASURES ALONG THE AXIS OF MOTION OF BODY "i" (ONE OF THE TWO IS VARIABLE)

Fig. 8-3. Manipulator joint coordinates and parameters

equal or proportional to the output load. These force-reflecting systems are called bilateral control system as compared to unilateral control systems where output directly follows input. Force-reflecting bilateral control systems can be combined with position control systems.

Master-slave manipulators may use either the unilateral control system or pure position control systems (Figure 8-4) or some combination of position control and force-reflecting control (bilateral control system) (Figure 8-5). These combination position-control/force-reflecting control systems are capable of using any type of manipulator powering in conjunction with any type of master-slave coupling.

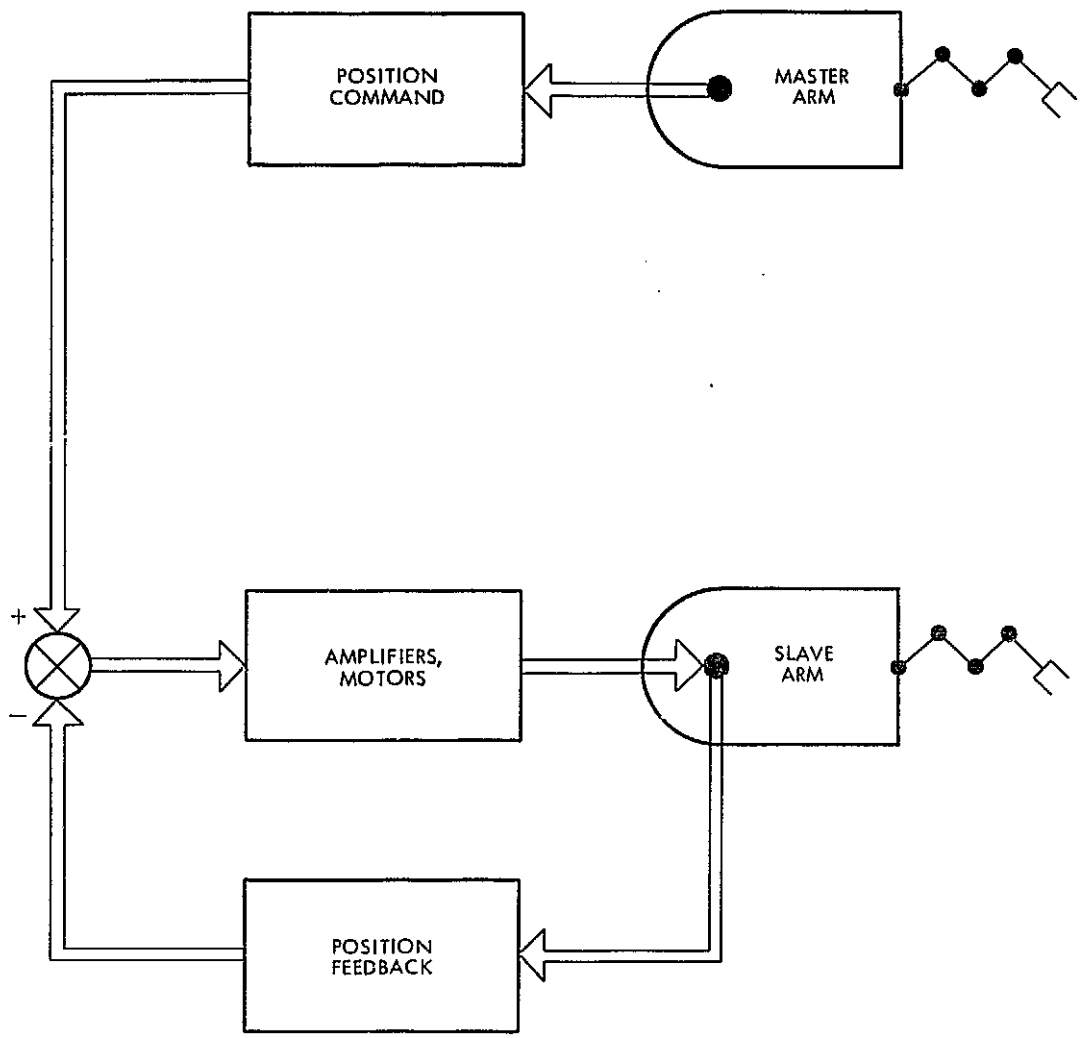


Fig. 8-4. Unilateral master-slave control scheme

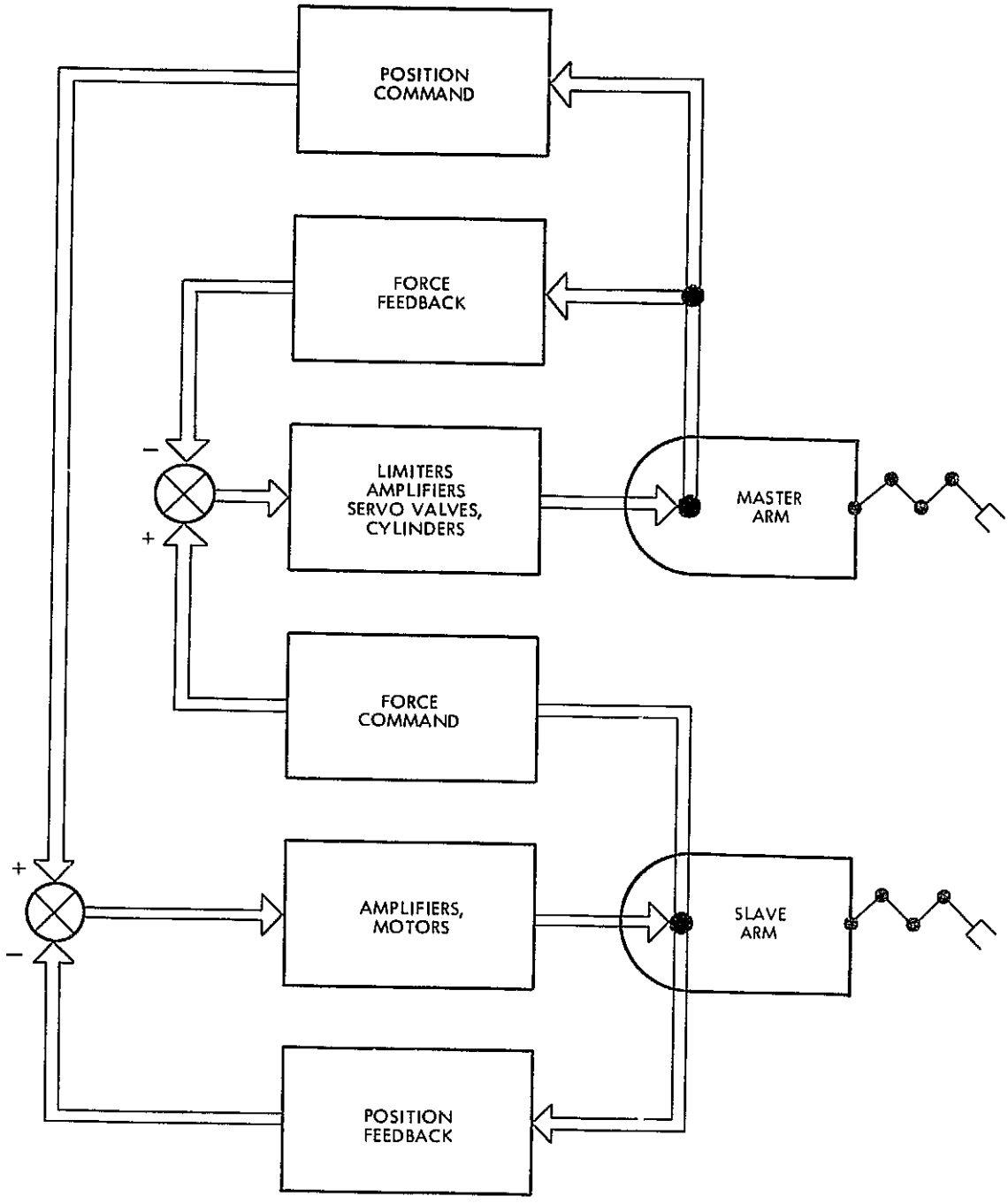


Fig. 8-5. Bilateral master-slave control scheme

Rate control is generally used in master-slave manipulator systems when forces to be applied and loads to be handled are in excess of a person's capability. In rate control these forces are not reflected and so the operation is not tiring to the human operator. Rate controlled manipulation systems are

best suited for grasping, holding, moving, orienting, and force applying operations. Sensing of forces, hardness, etc. can be determined indirectly by specific means designed into the system. The main deficiency in rate controlled manipulators is in the guiding operations where manipulative paths other than straight lines are to be followed. In a manipulation system with only rotary joints (non linear) even following a straight line can be extremely difficult for a human operator.

A specific type of master-slave manipulator system is the exoskeleton controller. This type of controller varies from a total sleeve type of controller arm to as little as a pistol grip.

In exoskeleton type manipulators, the positioning of the slave portion is adjusted to conform to the positioning of the master portion of the system. The advantages of this type of control are immediate feedback of slave position and orientation, force-feedback can be easily incorporated, and two independent manipulator systems can be simultaneously controlled. This type of system usually is able to provide fast performance in manipulative operations. Disadvantages to the exoskeleton type of system include the operator fatigue factor and the necessity of having large amounts of operating space.

Switch box or push button controllers are simple, low cost, low volume and flexible devices. They usually have a simple analog-type feedback controller for positioning. However, this type of controller has two significant disadvantages. It is very difficult to simultaneously control two or more joints (much less two separate slave armsystems) and they are really not suited for using force feedback in the arm control.

Joystick-type control systems are designed to overcome the switch box problem of simultaneously controlling two or more degrees of freedom in the manipulator system. This type of controller generally has proportional rate control which makes it very useful in making small changes in manipulator position. They can be used in conjunction with switch box controllers, extending the controllers coordinating capability to many different manipulator functions.

Combining a joy-stick controller with a programmable computer has led to a new class of control systems termed semi-automated. One type of semi-automated system developed at Charles Draper Laboratory, Massachusetts Institute of Technology (Ref. 13) lets the joy-stick control the rotational and translational axes while the computer resolves the linear inputs to follow the direction of the terminal effector. This type of arrangement may be used in a manipulator system using any number of degrees-of-freedom. A disadvantage of the joy-stick control system is the difficulty in using two manipulator systems simultaneously.

Current technology development is occurring in the semi-automated control area. Much on-going research is centered on the development of location and pressure sensors on line with the computer. This type system allows the human operator to move the manipulator near the desire object and then allows the computer control to take over and grasp the object with a predetermined pressure. This eliminates the guess-work involved in grasping an object and controls the pressure exerted on the object.

Programmed controller systems eliminate the step-by-step detailed control that manual control systems require of the human controller. The human operator is not an active element of the control loop as the system is guided by a "program". The human operator interacts through the program. The program is essentially a record of manipulations required to perform some function (point-to-point or continuous path). The required manipulator motions can be reproduced from the record through the use of servo mechanisms in the manipulator reacting to the recorded motions. These are "memory" controlled or "numerically" controlled systems. In point-to-point programming, the manipulator paths between the points are not specified by the program; the servo mechanism control will then servo the arm only according to the point inputs. This type of programming produces a very jerky motion in the manipulator. If smooth, continuous motion is necessary or desirable, a magnetic tape capable of storing many thousands of closely placed steps can be added to the control system. Continuous path programming guides the manipulator across a prescribed trajectory. The servo control forces the arm motion to the prescribed trajectory, which necessitates the control of time-position relations.

The point-to-point control mode is obviously simpler, however many applications require the precision of continuous path control.

There are several ways to produce a "program" for manipulator control. The "teach-in" technique is widely applied whereby the arm is physically moved by a human operator from one required point to another, or through a required path. Potentiometer read-outs from the arm joints corresponding to the points or the path are then recorded on tape. This type of "programming" is very suitable for repetitive operations. The associated servo controls can also correct for disturbances in the operation. However, for new operations the arm must be completely reprogrammed.

These memory-control types of "programmed manipulators" (together with their servo controls) do not have "perception" and "decision making" capabilities. They do not exhibit "machine intelligence" because they cannot interpret the conditions of the work and its environment, and act accordingly to perform a required task. A machine (a manipulator-sensor-computer system) that could sense external data and derive its own course of activity accordingly through a general program would be a true robotic arm with "machine-intelligence" capabilities.

In programmed manipulator control systems the general desire is to provide as much operational autonomy to the manipulator as possible in an appropriately integrated man-machine control environment. A primary goal is to construct general control programs for manipulator-sensor-computer systems which will enable the manipulator to interpret its own work and the related task environment, and make decisions accordingly to perform tasks stated in general terms by a human operator.

The addition of computers makes it possible for higher levels of control than the "teach-in" system is capable of performing. It is possible to move the manipulator along straight lines or in other geometric paths using an external rather than internal type of coordinate system. This allows the manipulator to arrange objects in regular arrays or to interact with objects moving at a constant velocity. This ability to assimilate the appropriate drive signals to the servos from the workspace coordinates is known as resolved-motion-rate control.

This ability to transfer control of the manipulator from direct human control to pre-programmed computer control has been accomplished by establishing a "hierarchy of subproblems" (Figure 8-6). This allows each level in the control hierarchy to accept commands from the next higher level and transmit ordered sequences of commands to the next lower level, using sensory feedback to close control loops where appropriate. The sensory feedback indicates the state of the manipulator and the environment. Sensory inputs include description of the position and motion of joints, or information from proximity, force or touch sensors on the terminal effector. Feedback data may also input timing signals from external equipment with which the manipulator must interact. The feedback data may additionally describe the location of obstacles, shape and location of work pieces or equipment and the motion of reference coordinates. At higher levels of control the manipulator may require the ability of human perceptual senses such as optical, tactile, or acoustical. The control of the manipulator requires that each level of control in the hierarchy be able to transform commands from a higher level into command sequences for the next lower level. This requires an immense amount of feedback data which is necessary in these computations. The amount of data needed varies proportionally to the degree of uncertainty in the environment.

There are several strategies by which sensory feedback data can be used in different levels of the control hierarchy to compensate for these uncertainties. One method is to determine the exact position of the objective and then translate, rotate or stretch previously determined trajectories to compensate for measured positional errors. A second method involves introducing the feedback data into the equations used in calculating the terminal effector trajectories. A third technique involves an adaptive system using a distributed storage system to learn or store the correct output for each relevant combination of inputs. This type of system can cope with large numbers of variables (both command and feedback) and exhibits many of the characteristics of a conditioned reflex.

If there are large uncertainties in the realm of the manipulator then more complex feedback information and control techniques are necessary. If the objects of interest are close together or randomly placed, many times force, touch and proximity data are not sufficient to perform the task. To

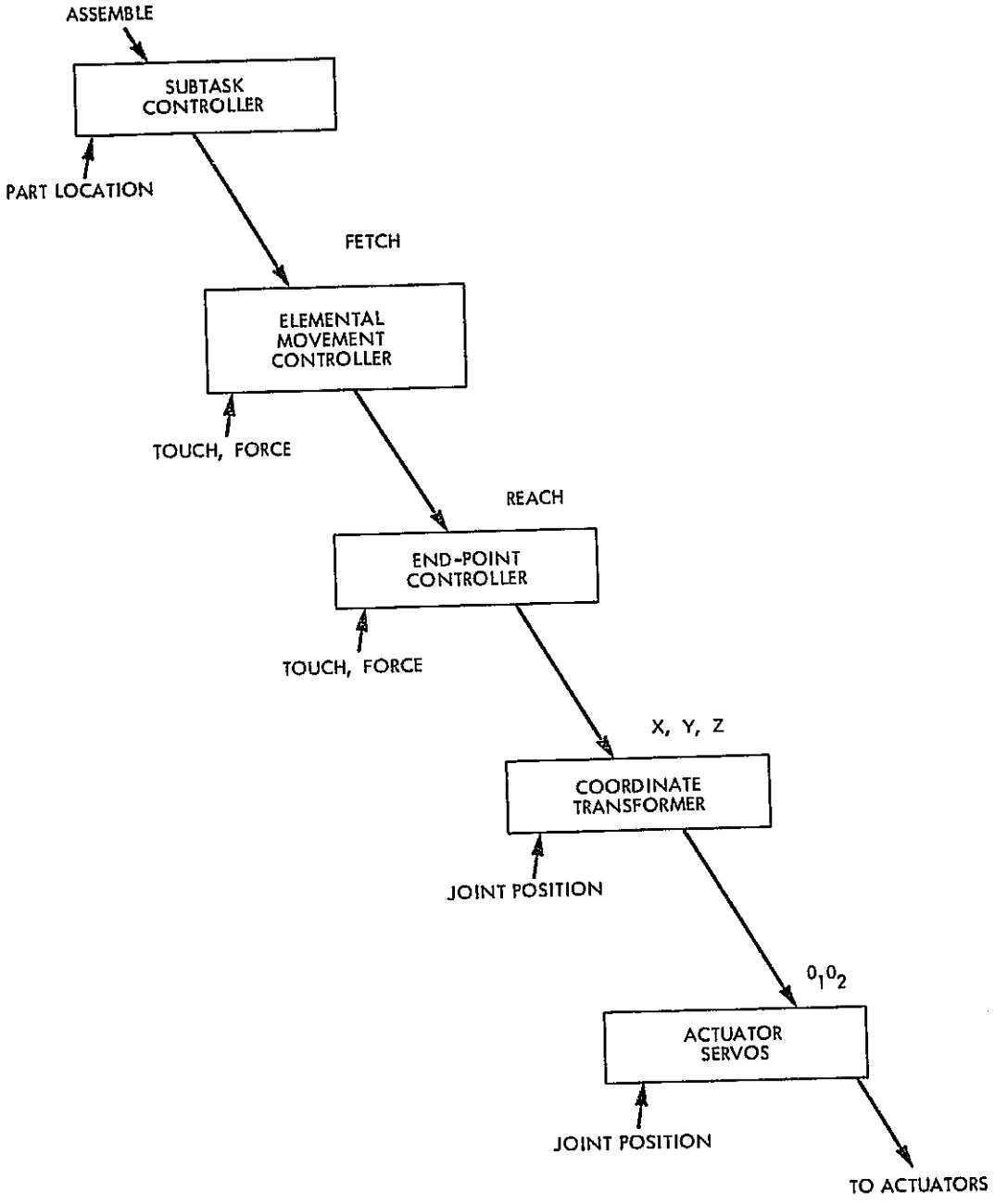


Fig. 8-6. Hierarchy of control levels

handle situations of this type, the manipulator must be endowed with a "knowledge frame" or internal data structure that represents the state of the environment to the manipulator in a manner which is meaningful. Additionally the system must contain sufficient sensors and processing circuitry for analyzing the environment and keeping the system up to date on environmental conditions.

There are several techniques currently being studied which deal with obtaining the feedback information necessary to perform this level of activity (Ref. 13, 15).

One system takes data from a television scanner and processes it to find edges, define shapes, analyze shapes and set up data structures to identify how these data points merge into a meaningful description of a real object.

The use of lasers has also been incorporated in imagery or teleoperator systems. One system at the Stanford Artificial Intelligence Laboratory uses lasers to illuminate objects. Where the plane of the light intersects the object contours are determined. By changing the plane several times a composite 3 dimensional image of the object is developed. The Jet Propulsion Laboratory is developing a system which may be used for locating and picking up rocks in a extraterrestrial rover type of vehicle. The system utilizes a television camera and an analysis program to detect the outline of an object (a rock) and then uses a laser range detector to measure the objects position and distance within the realm of the manipulator.

There are several types of actuator systems available for use in manipulator systems as depicted in Figure 8-7. The type of actuating power used generally depends on the application and specifications of the system.

Other aspects to be considered in a manipulator system include workload, work space and load lifting capability. The workload of a manipulator system is the load to manipulator weight ratio and is generally expressed as a fraction of its own mass.

The work space defines the reach out capability of the manipulator can be described in rectangular, cylindrical, or spherical coordinates, depending on the design of the rotary-linear joints for that given manipulator.

The load lifting capability describes the amount of mass that the manipulator is able to handle.

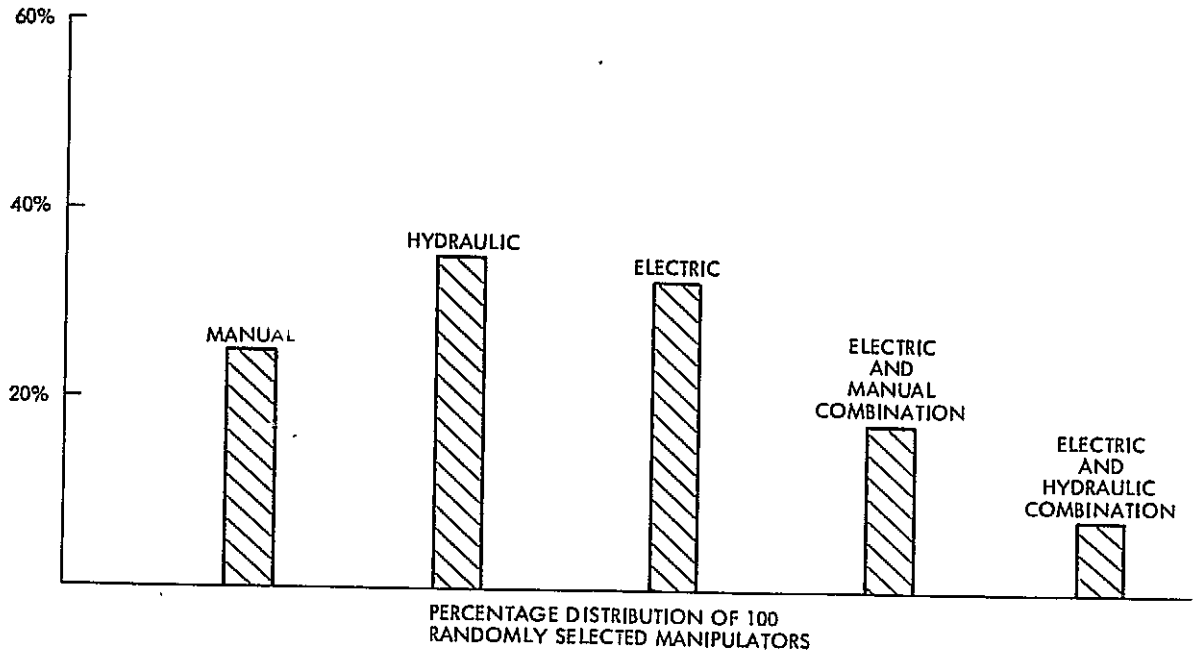
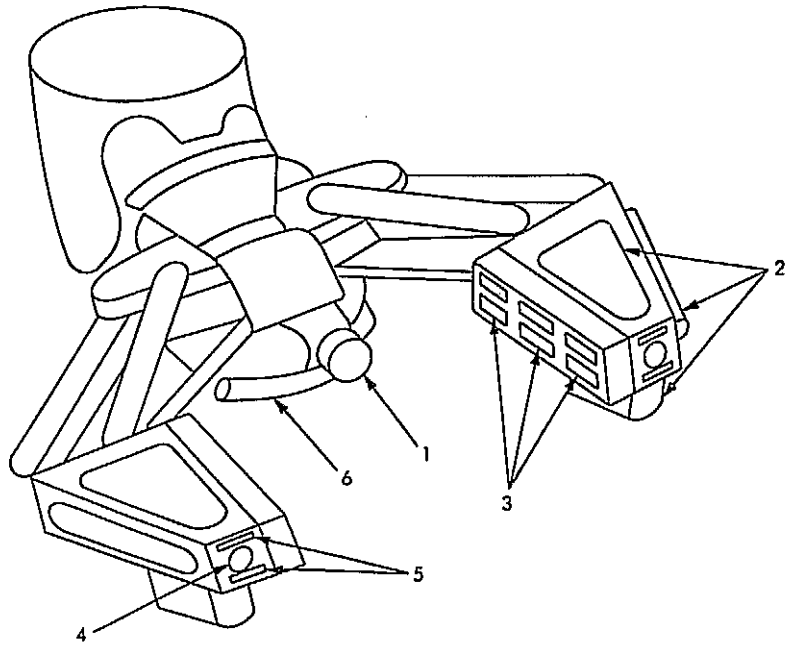


Fig. 8-7. Manipulator actuator systems

Terminal effectors or end devices provide the interface between the manipulator and the objects to be handled. They can be functionally compared to the human hand and fingers. However, the capabilities of existing terminal effectors cannot in any practical sense be compared to the dexterity and sensitivity of the human hand and fingers. There are two groups of effectors, laboratory and industrial terminal effectors and prosthetic devices.

Laboratory and industrial effectors are usually custom made, specifically designed for a given task. Most haveonly two "fingers" and could be called grippers. Mechanical fingers are usually called jaws. The two most common types of hands have either parallel or swinging jaws (Figure 8-8).

The shape of the applied jaws can be straight, linear segments forming a convex contour, curved (tong type), or a combination of linear and curved segments arranged into a convex shape. Tweezer-like spring jaws are usually used for handling small delicate objects, while wide faced jaws are generally employed for handling soft materials. In some cases the "fingers" are supplied with "tips". The jaw "hands" can also be supplied with jaw surfaces (rubber, aluminum, etc.) to match the work they will do.



THE SENSE ELEMENTS ON THE HAND

(1) SWITCH CLOSING IF TOUCHED; DETECTS POSITION OF OBJECTS BETWEEN FINGERS; BINARY OUTPUT.

(2) A TOTAL OF SIX CONTACTS (2 PER PLATE) CLOSING IF TOUCHED; INDICATE CONTACT WITH FINGER SURFACE; BINARY OUTPUT.

(3) 6 PRESSURE ELEMENTS IN RUBBER PAD, DETECT FIRMNESS AND LOCATION OF GRIP; CONTINUOUS OUTPUT (VARIABLE RESISTANCE).

(4) PHOTODIODE; REACTS ON SHADOWS CAST BY BLACK OBJECTS; CONTINUOUS OUTPUT.

(5) 2 PRESSURE PADS AS IN (3).

(6) PRESSURE ELEMENT ON BOTTOM OF WRIST; CLOSES WHEN HAND RESTS ON TABLE; BINARY OUTPUT.

ELEMENTS (2), (3), (4), AND (5) APPEAR ON EACH FINGER.

Fig. 8-8. Typical mechanical hand with sensors (MIT)

The "hands" can be single action, in which a movable finger pushes and holds the object against the second stationary finger, or double action, in which two moving fingers close simultaneously to grip the object. In some cases the two fingers can be controlled independently. In a NASA design (Ref. 13) the independent control of the two fingers is "reflexive", actuated by toggle switches placed on the inner part of the fingers; if one finger comes in contact with the object, the toggle switch stops the motion of this finger, while the other finger can still move to contact the opposite side of the object if the two fingers did not contact the object simultaneously. In some cases different terminal effectors are used for vertical grip and horizontal grip. There are also terminal effector designs where the jaw "hand" can perform small linear motion outward from the "wrist" to adjust the hand position relative to the object without moving the whole arm complex.

A jaw "hand" is normally used as a tool in itself; it is used in picking up and transporting things, or turning bolts and nuts, i.e., applying torques. In some cases a jaw "hand" is also used as a clamping device for holding other tools. As an alternate to holding tools in a jaw "hand", there are some designs where the jaws can be removed and tools attached in their place. This interchanging of hands or tools can be difficult. However, in one design, made by PaR systems, the hands themselves are remotely interchangeable by use of a fixture and the motions of the manipulator, (Ref. 14).

In most jaw "hand" designs, provisions are made to adjust, vary, and limit the gripping force. All jaw "hand" designs provide static load holding ability. The developed jaw "hand" control and drive systems employ a variety of technical solutions: mechanical, electrical, electromagnetic, pneumatic and hydraulic. Current study is involved in developing jaw "hands" which also incorporate simple tactile or pressure sensors attached to the "fingers". An example of the types of sensors which may be incorporated directly into a mechanical "hand" is shown in Figure 8-8.

To apply high gripping forces, or to handle very heavy loads, hook and anvil type "hands" are used. (The usual solution is that a hook is affixed with a power-driven anvil.) Some "hands" have also been built with two hooks and an anvil to obtain some spread on the object.

A great many special hands have been built for industrial applications, or specific laboratory requirements. However, very little work has been done to develop hand/finger systems with some general (dexterous) capabilities to match a variety of object handling or tool using requirements. One reason may be because there are obvious problems associated with the construction, use and control of mechanical "hands" that are more dexterous and articulated that the commonly applice two-finger terminal effectors. Preliminary trade-off studies (Ref. 13) indicate, however, that development work toward more dexterous and articulated mechanical "hands" would have considerable pay-off in several applications. The research necessary to develop more dexterous and articulated mechanical "hands" may come in the area of prosthetics.

In the last 10 to 15 years considerable effort has been made in the field of prosthetics to develop externally powered artificial hands which could compensate for some of the dexterities of a human hand. In prosthetics, the artificial devices are designed to meet needs of the human body, and to conform with the wishes of the amputee. The use of these two design philosophy rules have produced interesting approaches and results in artificial humanoid hand design.

One such hand design (Ref. 13) exemplifies an interesting solution to man-machine interface problems relating to control and operational requirements of end effectors. The design introduces internal (local) feedback through pressure sensing to control the grasping motion and/or the prehension force. The internal (local) feedback (or automatic control) relieves the "controller" (the patient) of some decision making, and decreases the dependence of the "controller" (the patient) of some decision making, and decreases the dependence of the "controller" on visual cues to make control decisions.

8.1.3.2 Quarantine Considerations. The conceptual design of a remote manipulator laboratory system should be based on two premises: (a) that maximum safety will be attained by containing all hazardous biological material within an impervious barrier, and (b) that maximum output will be achieved in a system in which the design parameters of the physical elements are derived from normal behavior characteristics of man. Solid barriers can be built with standard materials and fabrication methods. Arrangement of barriers so that

work is not impeded is difficult, if not impossible. Impairment of function can be minimized by designing a system which conforms to human characteristics. This requires closer attention to "human factors engineering" than is habitual among design engineers. It is essential that the human factors be given equal weight with hardware considerations.

In a remote manipulator system, the human operator and the machine he controls should be considered as the two parts of one overall system. The machine assemblage will include controls by which the man can influence its performance, and displays, such as lights, moving parts, visual and auditory signals which transmit information to the man. The manipulator is also acted on by other elements of the environment (input) and produces an output. Input may be information or materials, and output may be material objects or the performance of some action. In the design of a manipulator system, the problem is to adapt the machine elements so that they function effectively with the human elements.

Several requirements are immediately apparent. Automated control systems do not seem to possess the flexibility and adaptability necessary to handle biohazardous material.

Currently available programmable manipulator systems do not have adequate perceptual and decision making capabilities. As previously mentioned much work is being performed in the area of machine or artificial intelligence. Using techniques such as the "hierarchy of subproblems", "knowledge frame", and visual imaging systems, manipulator systems or robots are able to see, feel, and "understand" what is happening in the immediate environment. However, while it is nice to allow the machine to make elementary decisions about color or shape, it is a substantially different problem to allow total autonomy when handling extraterrestrial or any other hazardous material.

Additionally, the machine must not make excessive demands on the man. Incoming signals or stimuli must be of such nature that they are easily perceived. Control actions must be simple and within the speed and force capabilities of the researcher. Most importantly, if training is to be minimized and speed of operation maximized, the central nervous system functions should

be kept as simple as possible. This means that the relation between control action and machine action must seem natural to the man. He must not be called on for complex judgments or difficult estimates of position or velocity in the course of controlling the machine. Finally, the usual requirements for comfort and avoidance of fatigue apply.

Psychological considerations are very important. Any device which introduces a functional link between the operator's hand and the work piece is a potential source of frustration. The operator will be motivated to accept such functional links if they clearly add more to his powers than they detract from his sensations of immediate perception and control. A principal objective of the system design is to provide a sufficient feeling of enhanced power to the operator to override the frustrating effects of the barrier and manipulator interposed between him and his experiment.

The system must be capable of carrying out normal biological laboratory operations with a speed and effectiveness as nearly as possible equal to performance using the gas tight glove box system. This means that work and storage spaces must be adequate and must be arranged in logical order. There must be means for readily moving materials, including animals and instruments, within the system enclosure. There must also be convenient means for passing objects through the enclosure barrier without hazard. Manipulators, conveyors, and other mechanical handling equipment must approach the speed and precision of the human hand and arm. Visual, tactile, and auditory information from objects and operations within the barrier must be transmitted to the operator without distortion or attenuation.

The system must be "fail safe." No emergency should impair the system barrier. Moving parts should lock in the event of power loss or the sudden incapacitation of the operator. Moving elements should be fitted with interlocks or limit switches so that destructive impacts and spills will not result from operator errors.

Equipment must function for long periods without maintenance. Elements within the enclosure must be decontaminated prior to maintenance or repair. This adds materially to down time. It should be possible to decontaminate and remove subassemblies without shutting down the entire system.

Materials of construction should be selected to withstand the usual decontamination procedures.

The solution appears to be a semi-automated system with a manual override. Using the available sensor systems a feasible, practical manipulator system could be envisioned. The system will incorporate two overhead, bilateral master-slave manipulators with optional computer control. This allows the optimal use of the sensing mechanisms in determining distances to objects, grasping, forcing, and orienting. Each system should have one large manipulator for heavy objects and a "mini-manipulator" for lighter weight and more precision work.

Each manipulator will consist of two arms to provide the same abilities and dexterity which the researcher normally has. Each isolated manipulator system should have redundant manipulator capability. Each arm should have a minimum of nine degrees-of-freedom plus various terminal effectors. This includes three translational, three rotational, and three end fitting actions.

Use of several types of terminal effectors will enhance the dexterity and adaptability of the manipulators. The effectors or "hands" should be the conventional laboratory or industrial type of hand. The more elaborate prosthetic device with its concomitant complexity are not necessary for this type of work.

To perform the following manipulations, at least seven different types of "hands" are required. All seven effectors should be interchangeable between the two arms of a manipulator. Three sizes of independently controlled or reflexive, two-finger grippers will be required. For animal work special two and three jawed "hands" will be necessary for holding the animals. A syringe driver (a modified two-jaw clamp) will also be necessary for injecting and may be used for pipetting.

A turret type of hand which can accommodate several instruments (i.e., scalpel, forceps, scissors) will be needed.

The use of electrical power makes for the easiest adaptability for use in conjunction with an absolute barrier system. However in the event of power

failure, the arm will be rendered useless. Use of hydraulic power is feasible, but there is the problem of fluid leakage. Mechanical connections are the most tiring for the researcher but provide the most reliable type of manipulator system.

A combination of electrical and mechanical would supply the ease of electrical power with the fail-safe and over-ride abilities of manual power. The work load capabilities of the manipulators does not appear to be a problem. Only relatively small amounts of mass will be maneuvered (under 2 kg) at any one time by the "mini-manipulator" while the larger manipulator should be capable of loads up to 50 kg.

Discussion of the procedures and requirements for the handling of returned soil samples by necessity is very general, as they have not been firmly established. As a general guideline, the summary of Lunar Quarantine Biotest Protocols (MSC 03261) (Ref. 16) December 4, 1970 was used. This document includes applicable sampling procedures in the following areas: direct observation protocol, bacteriology/mycological protocol, virology and mycoplasm protocol, mammalian vertebrate protocol, invertebrate and fish protocol and the botanical protocol. As an example of the types of procedures necessary to perform quarantine investigations, the summary of Bacteriological Mycological Protocol is included in this report in para. 8-2.

To perform the activities summarized in the lunar protocol mentioned above, it will be necessary to perform the following manipulations:

1) pick up small objects
2) operate pipette
3) operate inoculating loop
4) operate syringe
5) handle petri dish
    a) lift and replace cover
    b) pour plates
    c) streak
    d) count

6) handle test tubes and flasks

   a) shake
   b) pour
   c) remove and replace screw caps
   d) remove and replace cotton plug

7) weigh materials on micro-balance

8) handle microscope slides

   a) smear
   b) stain and fix
   c) examine

9) operate mechanical equipment

   a) blender
   b) electric mortar
   c) electric mixer
   d) electric sample crusher
   e) sonicating device
   f) centrifuge

10) operate filtration devices

11) putting samples into and removing from equipment

   a) centrifuge
   b) incubator
   c) autoclave
   d) sonicating devices
   e) refrigerator
   f) deep freeze

12) use of instruments

   a) forceps
   b) shears
   c) dissecting instruments

13) handle ampules

a) open
b) fill
c) seal

14) handle animals

a) catch and hold
b) inject
c) bleed
d) temperature

Equipment

The equipment and instruments necessary to perform the quarantine analysis or any other biological study is dependent on the specific tasks to be performed. However, there are many pieces of equipment which are used in standard practice. It is anticipated that in the event a manipulation system is constructed, certain pieces of equipment will be incorporated in the manipulator containment system design.

Equipment which should be an integral part of the total system includes: autoclaves; gas and steam; incubators; anaerobic and aerobic; sample sonicating devices; centrifuges; microscopes, light and fluorescence; refrigerator; deep freeze; and plate counters.

Additional equipment (based on the L. R. L. protocols) includes, but is not limited to: automatic pipetter, electrical mixer, electrical mortar grinder, electrical sample crusher, scales, balance, filtration devices, and Bunsen burners.

If animal work is to be performed, special provisions for cages, animal boards, squeeze cages, etc. will have to be made. If botanical work is to be accomplished similar provisions will have to be made.

A matrix has been prepared (Table 8-1) to some representative master-slave type manipulator systems against a list of general characterizations or criteria. The decision to use a manual or semi-automated control system rather than a totally programmable system contributed significantly to the brevity of the list.

Table 8-1. Characteristics of Some Remote Handling Systems for Quarantine Application

Characteristics	Koelsch	MBA Arm	Ames Arm	Curvlinkage	Brookhaven	PaR 3000, mini-manip.
1. Sterilizability	Electrical may be modified	May be able to adapt	No steam; potentiometers and motor rated to 120-130°F-bearings to 150°	No steam	Same bearings as Ames arm	May need special lubricants
2. Cleanability	Yes	Yes	Yes (Geardriven cleanlines may be hard to maintain)	May be problem w/detergents	Yes	Developing stainless steel (easily cleaned)
3. Particulate generation	Sealed Systems	Sealed Systems	Gears sealed	Sealed Systems	Not all bearings are sealed	Sealed Systems (Uses bellows to pressurize sleeve and maintain)
4. Performance of Unique Bio-procedures	Used for handling larger samples and equipment	Will be designed for specific requirements	Good for 1/4" wide 1" long; 8-D. C. motors positional servo in arm;	No feedback from potentiometers	M-S force reflecting bilaterals; lift up to 10 Kg	Can pick up a needle and up to 5 lbs.
5. Degrees of Freedom	6 + T. E. *	6 + T. E. *	8 + T. E. *	6 + T. E. *	6 + T. E	7 + T. E. *
6. Biobarrier Interface Problems	Need seal around hydraulic line	None-electrical control or partial hydraulic	None-electrical control	Hydraulic - need seal around line	None-Hermetically sealed for complete isolation-elect. connection	None-use bellows to maintain press. of system
7. Manual vs. Automated Control	Manual push button-finger motion is hydraulic	Manual-M-S sleeve type	Sleeve-type Manual or auto system may be semi-auto. using sensors	Joy-stick; rate; position control-manual, auto. Man. to position arm near object; semi-auto use sensors for final grip	Man. force reflect. manip. bilateral	Joy-stick - semi-auto; can set grip press 0-200 lbs. elect. control, bilateral
8. Operation in Weightlessness	Yes	Undersea use	Yes	Yes (Question in vacuum)	Yes; no counter balance problem but may have bearing problem	Yes

*T. E. = Terminal Effector

Table 8-1. Characteristics of Some Remote Handling Systems for Quarantine Application (Contd)

Characteristics	Koelsch	MBA Arm	Ames Arm	Curvlinkage	Brookhaven	PaR 3000, mini-manip.
9. Assayability	Yes	Yes	Yes	Yes	Yes	Yes
10. Future Availability	Yes; commercial product	Commercial-can devise system for specific requirements	4 units currently	Used by U. S. Navy	Specially designed system	Yes; commercial
11. Proprietary	Yes	Yes	NASA Ames No	U. S. Navy No	Now yes - based on Brookhaven design	Yes
12. Operator Training	1-2 mo. 1 hr/day	6-12 Mo, 1 hr/day	1-2 months 1 hr/day	1-2 months 1 hr/day	Operator 75% efficient after 3 min.	1/2 to 4 hours
13. Cost	Depends on requirements	Depends on requirements	35-50 k/copy + comp. console	50K and comp.	300 k for 1st pair of arms	6k to 80k - depends on size and requirements
14. Failure Modes	Lock up mechanism and dual pump hydraulic power	Lock up system available	Not fail safe - can't overdrive it; built-in mechanical stops	With hydraulic leak may lost 1 joint - may guide sys. down safely	Fail safe sys. locks after 30 millisecs	Fail safe - locks w/power failing; can remove arm and make adjustment w/other arm

*T. E. = Terminal Effector

None of the manipulator systems discussed can take heat sterilization in their current state. The PaR systems may be the easiest to modify with only a change in lubricant type being necessary for application of dry heat sterilization techniques.

All of the systems are cleanable using standard cleaning techniques. However, there may be a problem with the use of detergents on the Curve-Linkage manipulator. PaR manipulator systems are developing a stainless-steel arm which will be easy to clean.

Cleaning the manipulator is virtually meaningless if the arms are self-contaminating by generation and shedding of particulate materials. All systems with the exception of the Brookhaven are either totally sealed or the gear mechanisms are sealed in some manner. In the Brookhaven, however, not all of the bearings are sealed. The PaR system uses bellows to pressurize a covering sleeve to maintain a sealed environment.

The ability to perform unique bioprocedures is a catch all category which includes the types of manipulations previously enumerated. Handling of masses up to 5 kg is easily accomplished by all of the listed systems, except for the PaR mini-manip. The mini-manip is useful for only light weights (up to 2-1/2 kg) but has more control in detailed operations.

The electrical control systems adapt very well for use with barrier systems. Use of hydraulic power (Koelsch, MBA, CurvLinkage) presents problems in achieving total isolation between the two sides of the barrier. Use of a bellows system such as that employed by PaR to maintain pressurization may be used to solve this problem.

All of these manipulators use the manual or human type of control. Several (MBA, Ames, Curv Linkage, PaR) have been adapted to use some computer control and feedback making them semi-automated systems as desired. Both MBA and Ames employ "sleeve" type of controllers. This concept is not as practical as the other control types as it limits functional performance of the slave to the reach of the researcher. Systems such as the Brookhaven, PaR, Koelsch and CurvLinkage can be built with different reachout capabilities to provide the necessary handling ability. All except the Koelsch have the necessary bilateral control system.

The CurvLinkage has three million possible combinations of controlling mechanisms. It has been adapted to be manually moved near the desired object and then using the semi-automated capability grasping the object using the sensors for guidance. Using the PaR, the grip of the "hands" can be preset to a given pressure between 0 and 200 lbs. A combination of these two features would be very useful.

All of the manipulators are capable of being used in a weightless condition such as space. Actually, the elimination of maximum load constraints is a distinct advantage, allowing the smaller, lighter manipulators to perform the functions of the larger types. However, CurvLinkage may not be able to function in the space vacuum. Additionally the Brookhaven may have to use a different type of bearing in a weightless configuration.

The redundant, double arm system previously described allows for the assaying of the manipulator/barrier system by itself to insure maintenance of the pristine state of the sample material. All of the surveyed manipulators possess this capability.

The two criteria of future availability and proprietary aspects can easily be combined. The Koelsch, MBA, CurvLinkage and PaR manipulators all are commercially available. The Brookhaven was originally designed at Brookhaven National Laboratory; however, the design is now being marketed by a private company. All of these systems are available and may be modified to meet the requirements of the customer. The Ames and CurvLinkage manipulators were designed and built by organizations of the U. S. Government. They have been produced only in limited quantity, and are not commercially available.

The question of length of operator training is very difficult to answer. Brookhaven's manufacturer claims that 75% efficiency may be obtained after as little as 3 minutes of training. MBA feels that six to twelve months are required to utilize the manipulator accurately. Training of the operator probably should take between two and four months, but only lasting one hour per day. This will eliminate frustration and fatigue in the operator, with the concomitant animosity cause caused by frustration. Use of a manipulator

does not just require hand coordination, but rather the whole body has to be trained to operate the manipulator as easily as one would his own arms.

The cost of a manipulator is totally dependent on the requirements to be placed on the system. The prices quoted in the matrix are averages, do not include any additional development costs, and especially do not include any computer or computer console costs.

The last but certainly not the least aspect to be considered, are failure modes. Any manipulator which does not have a fail safe mechanism to hold the arm in position with a loss of power is unacceptable. As noted in the matrix the Ames arm does not have a fail safe mechanism. The CurvLinkage, in the event of a hydraulic leak, may lose control of one joint but can be safely controlled back down to the rest position. All other manipulators lock up with a sudden power loss preventing potentially catastrophic events from occurring.

8.1.4 Conclusions

The conclusions of this study, based on the information available are as follows:

1. The master-slave manipulator concept is feasible for use in handling bio-hazardous materials.
2. Combining some sensory elements of a programmable control system to upgrade the master-slave to a semi-automated system would be advantageous.
3. The system should provide maximum safety for both the researcher and the extraterrestrial material.
4. Present terminal effector technology is adequate. However, they could be more effective with increased research.
5. It appears that manipulators are compatible with biobarriers.
6. The M-S manipulator concept can solve some of the problems discovered by the L.R.L. experience.

7. Development of manipulator systems will be substantially more expensive than use of existing glove box systems.

8.2 SUMMARY OF LUNAR RECEIVING LABORATORY BACTERIOLOGY/MYCOLOGY PROTOCOL

8.2.1 Objectives

1) To detect and attempt to isolate and identify, within the confines of this protocol, all viable micro-organisms isolated from sample material.

2) To contain all hazardous infectious micro-organisms isolated during the performance of the tests outlined in this protocol.

8.2.2 Procedure

8.2.2.1 Return Sample Distribution. The core (early) and pooled conventional lunar materials will each be subdivided into two parts as follows:

1) Core (early) Return Biosample

   a) Unenriched (Indigenous) cultures. Cultures media containing lunar material as the only source of nutrients.

   b) Enriched cultures. Culture media having known terrestrial additives as the source of nutrients. These will be blood agar, trypticase glucose yeast extract, and thioglycollate broth.

   c) Autotrophic and Nitrogen-fixing cultures. Culture media designed for growth of nitrogen-fixing bacteria or selected autotrophs will each be inoculated with 223 mg of sample material. A total of 4 different media, both fluid and agar-based will be employed.

2) Pooled Conventional Return Biosample

a) Unenriched cultures. Culture media containing sample material as the only source of nutrients.

b) Enriched cultures. Culture media having known terrestrial additives as the source of nutrients. These will be blood agar, trypticase glucose yeast extract, thioglycollate broth, Czapek Dox agar, Sabouraud dextrose agar, an extract in agar of 5 pooled terrestrial soil types, and six aquatic fluid, (water and mud) media.

c) Autotrophic and Nitrogen-fixing cultures. Culture media designed for growth of nitrogen-fixing bacteria or selected autotrophs will each be inoculated with 223 mg of sample material. A total of 4 different media, both fluid and agar-based will be employed.

8.2.2.2 Method

1) Add approximately 223 mg of sample material, of fine particulate size (<100 microns diameter), to each culture plate and each culture tube.

2) Use two types of exposure.

a. Particulate sample material

b. A buffered wash from particulate sample material

3) Prepare two different dilutions of each media, except indigenous agar, aquatic fluid, and pooled soil-type extract agar which have no dilutions, and incubate at various temperatures and under various atmospheric conditions.

4) Incubate all cultures, except pooled terrestrial soil-type sample and aquatic fluid media, at $4^{\circ}C$, $24^{\circ}C$, $35^{\circ}C$, or $55^{\circ}C$.

5) Atmospheric requirements are filtered air for aerobic organisms, a mixture of 10 percent CO_2 in air for microaerophilic organisms, and prepurified nitrogen gas for anaerobic organisms.

6) Label all media, tubes, plates and sample material.

7) Pour all test and control media into aliquots within class III cabinetry.

8) Test all culture media (agar plates and broth in tubes) for sterility by incubation at 35°C for 48 hours within the class III cabinetry.

9) Test samples of the prepared media for ability to support growth of the following control organisms: Staphylococcus aureus, Neisseria gonorrhea, Streptococcus species (β-hemolytic), Aspergillus niger and Haemophilus influenzae.

10) Prepare all media and reagents with deionized water certified to contain <1.0 ppm of extraneous ions.

11) Use each specified combination of medium dilution, temperature, atmosphere, and type of exposure with each, the core (early) and the pooled conventional return biosamples. In addition, use one uninoculated control with each combination.

12) Sterilize all materials (used and unused), supplies, and equipment out of the class III cabinetry or Sample Laboratory by autoclaving, exposure to ethylene oxide gas, or dunk tank containing sodium hypochlorite (5000 ppm available Cl^-).

13) Characterize and identify all isolates by morphological and biochemical techniques.

14) Identify and compare all isolates to the spacecraft hardware flora.

15) Take 35 mm color photographs to record colony characteristics and changes in media characteristics.

16) Summary of total requirements are as follows:

	Sample	Culture Plates	Culture Tubes	Culture Flasks
Core (early) return biosample	36.572 g	108	16	24
Uninoculated control		60	8	8
Pooled conventional return biosample	51.290 g	216	64	24
Uninoculated control		108	32	8

8.2.2.3 Observations

1) Record all observations in a logbook.

2) Record all observations made within class III cabinetry.

3) Examine media every 24 hours for the first three days and every 48 hours for the next 18 days for the following changes:

   a) Colony formation (size and number of colonies

   b) Turbidity

   c) Visual changes in the media

4) Compare all inculated media to control media.

5) Observe gross characterization of each different colony.

6) Identify bacterial/fungal types present by appropriate cytological and biochemical techniques.

8.2.3 Release Plan

See Return Sample Release Flow Chart for Bacteriology/Mycology.

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SECTION IX

STERILIZATION AND DECONTAMINATION TECHNOLOGY FOR SAMPLE RETURN MISSIONS (NASA NO. 193-58-64-03)

SECTION IX

STERILIZATION AND DECONTAMINATION TECHNOLOGY FOR SAMPLE RETURN MISSIONS

9.1 EXOTHERMIC COATINGS FOR SURFACE STERILIZATION

9.1.1 Subtask A Introduction

The objective of this subtask is to demonstrate and develop surface heat sterilization mechanisms for space hardware applications by the use of exothermic pyrotechnic (thermite) compositions. The rapid propagation and high reaction heat of thermite are capable to produce transient high temperature heating of the hardware surface to achieve sterilization. For return capsule application, it is further required that the interior part of the capsule should be maintained below the sterilization temperature, therefore, an adequate heat insulation mechanism is also required.

The advantages of the concept are easy to realize, it produces a uniform and complete heating of the surface including all microscopic cracks, it is simple in design and operation, and no sophisticated spacecraft (S/C) type of instrumentation is required. Thermite pyrotechnics have high energy densities; thus, the design is very weight efficient. Furthermore it can be operated independently of the environment, e.g., the ambient gas pressure.

9.1.2 Approach

Three approaches were investigated for the thermite coating sterilization mechanism: 1) open surface burning, 2) shielded surface burning, 3) linear column burning. The former appears attractive because the high temperature burning is achieved directly on the surface to be sterilized. In this case the mechanical and environmental requirements of the coating are stringent. The reaction must be self-sustainable under vacuum and the contaminants generated by the reaction have to be evaluated and minimized.

In the latter two approaches, thermite compositions either in the form of surface coatings or in the form of a mosaic of small diameter columns are imbedded in and protected by the metal surface to be sterilized. In this case, the fabrication technique, temperature profile, and heat insulation, need to be

demonstrated. Because of the insulation restriction, the amount of coating composition has to be minimized. The optimal coating thickness and burning rate required to maintain reliable propagation of the reactions will have to be determined.

9.1.2.1 Characteristics of Candidate Thermite Powders. Table 9-A.1 shows the compositions and burning rates of some typical thermite powders. These have been widely used to fabricate delay elements in explosive devices as well as heating elements in fuel cell batteries. Military standards for their production are well established. The burning rate is determined by the amount and particle size of the various ingredients. It is relatively insensitive to parameters such as loading density, ambient temperature, and pressure. For heat sterilization applications, a higher burning rate is more desirable in order to prevent possible failure due to heat loss and to simplify the heat insulation problem.

Table 9-A.1. Established thermite pyrotechnics

	% Composition	Burning Rate - sec/2.54 cm
1 $Zr(0.5\mu)/BaCrO_4$	55/45	0.05
2 Zr/Fe_2O_3/Inert	65/25/10	0.10
3 $Zr(8.0\mu)/BaCrO_4$	55/45	0.87
4 $Zr(8.0\mu)/BaCrO_4$	22.5/77.5	2.66
5 $Zr(8.0\mu)/BaCrO_4$	20/80	4.97
6 $Mn/BaCrO_4/PbCrO_4$	37/20/43	9.10
7 $Mn/BaCrO_4/PbCrO_4$	34/28/38	12.43

The chemical reactions of two thermite compounds can be seen in the following examples:

$$3\,Zr + 4\,BaCrO_4 \longrightarrow 3\,ZrO_2 + 4BaO + 2Cr_2O_3 + 376\ Cal/g$$
21% 79%

$$3\,Zr + 2Fe_2O_3 \longrightarrow 3ZrO_2 + 4Fe + 642\ Cal/g$$
46% 54%

Even though these heat outputs are considerably lower than those of pyrotechnics used for explosive device applications (typical value 1-2 K Cal/g), this amount of heat energy is sufficient to produce a temperature rise as high as 1000°C. These reactions produce insignificant amounts of gaseous products (<0.1%); therefore, they are pressure insensitive and self-sustaining under vacuum. When organic binders are used in the thermite coating formulation, the amount of gaseous products is increased. This increase in the level of contamination can introduce reaction propagation problems.

9.1.2.2 Ignition techniques. Ignition is the most crucial phase of pyrotechnic device reliability. The ignition temperature of thermite powders usually ranges from 400 to 500°C. Due to their low sensitivity as compared to pyrotechnic explosives, a longer heating (induction) period or a greater heating depth is required for ignition. For the tests using simple configurations, which were performed during this period of this study the following three techniques were used:

1) Direct hotwire ignition. 0.71 mm - diameter Tophet A resistance wire was imbedded in the thermite composition and actuated by a current ranging from 4-10A.

2) Hotwire - Boron pellet (B/KNO_3/Binder, 23.7/70.7/5.6). The assembly with the same type of hotwire was used for ignition in vacuum.

3) Hotwire - Thiokol X - 225 Igniter mix ($KClO_4$/Ti/B/Binder 72.3/14.8/6.9/6.0). This flexible assembly with the same type hotwire was used for ignition in ambient atmosphere.

For future tests as well as spacecraft applications a JPL squib can be modified for thermite coating ignition. This device uses a boron based pyrotechnic $B/KClO_4$/Viton 17/77/5 as the ignition match material, 50 μm-diameter 2.5 mm long Tophet A wire as bridgewire and a variable amount of $TiH_2/KClO_4$ mix as the output charge. The latter should be replaced by a thermite powder for thermite coating ignition. It is estimated that about 40 mg of the $B/KClO_4$/Viton mix in the squib header is sufficient to reliably ignite the thermite incrementally. When utilized this way all the desirable reliability features of the squib, which have been demonstrated in Mariner 71 and 73 and the Viking orbiter projects, will be present.

9.1.2.3 Linear Column Burning. Limited tests were performed to demonstrate this approach. Thermite powders with different burning rates were loaded at 1.35×10^8 N/m^2 of compaction into straight holes of different diameters in 5.08 cm - long aluminum bars having different cross-sectional areas. This geometry was chosen, because it is an element which can be employed to achieve the heating of a metal shell structure. While the loading pressure is typical of those used in explosive delay mechanisms, the hole diameters in the test bars were considerably smaller to assure safe operating conditions. The results are shown in Table 9-A.2.

Table 9-A.2. Results of linear column burning tests

Loading Pressure: 20 kpsi Al Bar Length: 5.08 cm			
Bar X-Section, cm	Hole Diameter, mm	Powder Burning Rate Sec/2.54 cm	Peak Surface Temperature, °C
0.635 x 0.635	2.0	4.97	Failed to ignite
0.635 x 0.635	2.0	0.87	120
0.635 x 0.635	3.0	0.87	260
1.27 dia	3.0	4.97	94
1.27 dia	3.0	0.87	113

The results indicate that reliable propagation can be achieved if a high burning rate powder and/or large column diameter is used. The mass of the aluminum bar did not appear to be a crucial factor affecting propagation. The thermal diffusivity of thermite compositions is much smaller than that of aluminum; therefore, the transfer of heat from the interior to the surface of the thermite column is the limiting factor for heat loss.

In practice, drilling long holes in the hardware and loading thermite powder into them are not simple tasks and need further evaluation to develop better techniques. A viscous flow or extrusion type of loading may be solutions to the problem. Spatial temperature distribution in a multi-column heating configuration has yet to be studied.

9.1.2.4 Surface Burning. To achieve mechanical strength and ease of fabrication, a binder is required for both open and shielded surface thermite coatings, even though the presence of the binder drastically affects the burning rate of the thermite powder. By using a high density, moderately sensitive thermite such as #3 listed in Table 9-A.1, the required binder weight percent can be reduced to 10% or less. A weight percent of 10 corresponds to a volume percent of 30 or less for typical low density organic binders. The densities of the thermite ingredients contained in the above compound are in the order of 6.4 g/cc for Zr and 4.5 g/cc for barium chromate.

Commercial resin Dapon M (diallyl isophithalate, density 1.64 g/cc, FMC Inc.), well-known for its high temperature resistance and strong mechanical properties, was used as the binder for the preliminary studies. The thermite powder and Dapon M powder were mixed with MEK solvent, then dried, pressed and heat cured on 5.08 cm - diameter aluminum sample disks. The disks had 0.5 cm - diameter central holes to feed through electrodes for direct ignition hotwires or installation of igniter assembly. For shielded coating tests, a second aluminum plate was mechanically clamped on top of the thermite coating to form a sandwiched type configuration.

Table 9-A.3 summarizes the results for the Dapon M - $Zr/BaCrO_4$ 55/45 mix as tested in open surface burning in air and vacuum and sandwiched by the 5.08 cm - diameter 0.635 cm - thick aluminum plates. 10 g of the thermite was used in the formulation and the coating thickness was about 1.5 mm.

Table 9-A.3. ($Zr/BaCrO_4$ 55/45) Dapon compositions surface burning results

$Zr/BaCrO_4$: 10 g Al Disc Diameter: 5.08 cm					
Dapon Weight, g	1.5	1.0	0.5	0.25	0.125
0.635 cm Al - Air Burning rate Sec/2.54 cm	X	14	3	2	1
0.635 cm Al - 0.635 cm Al	X	X	V 200°C	V 213°C	V 190°C
0.635 cm Al - Vacuum	X	X	X	V	V 109°C
Mechanical Properties	S	S	S	S	U

X = Failed to ignite
V = Ignited
S = Satisfactory
U = Unsatisfactory

The results indicated the following: an increase in the percent of binder reduced the burning rate; thus permitting greater heat loss. Both the sandwiched assembly and the vacuum test represented the most severe heat loss configurations. In a vacuum, the gaseous products generated by the binder tended to carry heat energy away from the burn front through a large plume formation. The heat loss effect due to the aluminum plate was also very evident. Post burn examination revealed that a thin layer of thermite coating in contact with the aluminum had not been burned due to insufficient heat. It appears that samples containing 0.5 g and 0.25 g of Dapon binder worked satisfactorally for the aluminum shielded and surface burning tests in a vacuum. However, it is felt that the 0.25 g of binder would be marginal for the mechanical strength requirements of an open surface coating.

The mechanical strength of the coating was judged by preliminary examination of its hardness and adhesiveness to the aluminum plates. In the future, some quantitative tests, such as pulling and impact, should be performed

to gain additional information. It is felt that a better mechanical configuration can be designed that will enhance adhesiveness. The temperatures shown in the table are the peak temperatures on the external surface of the aluminum plates measured by a copper-constantan thermocouple. A typical temperature profile is shown in Figure 9-A.1. The cooling rate depends on ambient conditions such as atmospheric pressure and the mass of the test fixtures attached to the plates. It is anticipated that several minutes of elevated temperature can be maintained on the plate surface.

Table 9-A.4 shows the effect on the peak temperature due to the scaling of the test parameters such as the coating and aluminum plate thicknesses. As the coating thickness increases and/or the plate thickness decreases, a higher temperature is achieved. However some non-linearity was observed. The 0.635 cm - 0.318 cm sandwiched aluminum plate assembly shows interesting data which may have hardware design applications. The 373°C of peak temperature rise on the 0.318 cm - thick plate is effective for sterilization. On the other hand the 200°C of temperature on the 0.635 cm - thick plate is below the melting point of a number of organic heat insulators such as Teflon, so that further heat insulation can be achieved by using these materials.

Attempts also have been made to use an active (flammable) binder, commercial crystal clear resin (density 1.1 g/cc, PPG Industries Inc.). Open surface burning under a vacuum on 5.08 cm diameter x 0.635 cm thick aluminum plates, as well as good mechanical bonding, was achieved with up to 1.0 g of this resin added to 12 g of the $Zr/BaCrO_4$ 55/45 thermite powder. Unfortunately, serious plume contamination was observed in the vacuum test. To date, the attempts to use inorganic binders and solid propellant binders have been unsuccessful, due primarily to contamination problems during burn.

9.1.2.5 Heat Insulators. In addition to the aluminum, which was demonstrated to be an effective heat baffle material, typical insulators, such as teflon, lucite and asbestos were tested. Disks made from these materials were clamped onto the thermite coated 5.08 cm diameter x 0.635 cm - thick aluminum plate. The firing tests indicated the following:

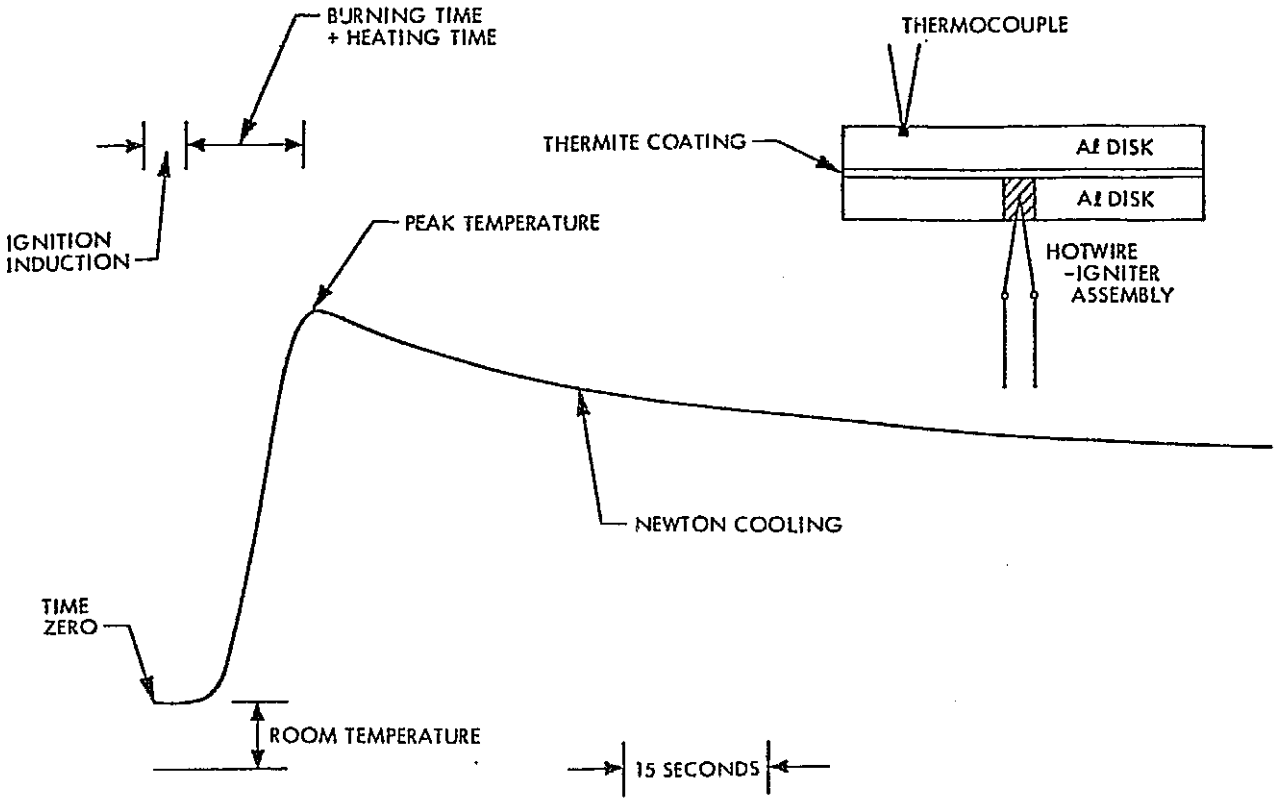


Fig. 9-A.1. Typical temperature profile

Table 9-A.4. Scaling effects

Boundaries*	(Zr/$BaCrO_4$ 55/45)/Dapon	Peak Temperature, °C
0.635 cm† - Air	10g/0.5g	190
0.635 cm - 0.635 cm	10g/0.5g	200
0.318 cm - 0.318 cm	5g/0.25g	164
0.635 cm - 0.635 cm	5g/0.25g	111
0.635 cm - 0.635 cm	20g/1.0g	299
0.635 cm - 0.318 cm	10g/0.5g	373 (0.318 cm Al) 200 (0.635 cm Al)

†Aluminum disc thickness
*Aluminum disc diameter = 5.08 cm; Pyro thickness: 0.7-1.5 mm.

1) The use of the insulators resulted in large increase in the peak temperature of the aluminum plate.

2) Organic insulators cannot be placed in direct contact with the thermite coatings due to severe vaporization of the insulation material.

3) The insulation effectiveness is controlled by the heat capacity of the insulator, i.e., largely by the insulator thickness.

9.1.3 Future Activities

During the next period, efforts will be primarily in the following areas:

1) Biological tests on the thermite coated aluminum plates to establish sterilization criteria.

2) Thermite coating burning on several hardware configurations. This is a necessary step to demonstrate the feasibility of fabricating shielded surface burning apparatus.

3) Continue the effort to develop a satisfactory coating for open surface burning by investigating the feasibility of using more sensitive thermite powders and binders to solve the plume contamination problem.

4) Improve the processing tests and techniques used in solvent handling and testing the mechanical properties of the various coating components.

9.2 SOIL STERILIZATION STUDIES

9.2.1 Subtask B Introduction

If it is assumed that the prime objective of a sample return mission is to acquire biological information, it follows that a nominal mission will dictate the return of an unsterilized sample to earth. If such a sample contains viable life forms it must be regarded as a potential hazard to terrestrial life forms and likely will not be permitted to enter the earth's biosphere until all U. S. /NASA quarantine requirements are met; therefore, sterilization of the sample must be considered as a contingency that might be dictated by an anomalous mission. In addition, it will probably be desirable to be able to sterilize a portion of the sample in order to make it available for other non-biological scientific investigations. A returned sample sterilization process that would enable the acquisition of maximum biological, organic, geochemical and morphological science information would be optimal.

Sample sterilization techniques must be examined in terms of their ability to kill organisms resident in soil. Soil having properties approximating those of the return sample would most appropriately serve as the menstruum for studying the resistance of bacterial spore forms of aerobes, and anaerobes to candidate sterilants. The gaseous atmosphere, including moisture and pressure, in which the soils are heated to establish resident spore heat resistance, should approximate anticipated conditions. For example, the most probable atmospheric pressure, humidity band and gaseous atmosphere within the sample return container are considerations when establishing sterization requirements. The effect of sterilization processes in relation to destruction of inorganic, organic and biological information are also of primary concern.

Culturing is the method of choice for demonstrating the presence of viable populations of microorganisms and thus determining the efficiency of a sterilant. Special purpose nutrient substrates have been developed to cover the broadest ranges of requirements for different species of microorganisms. The difficulty in developing a single nutrient substrate which will detect all viable microorganisms is pointed up by the fact that there is no uniformly satisfactory sterility test medium (e. g., Bowman, In Industrial Sterilization,

Duke Press, 1973). Whether all cells which are metabolizing can eventually reproduce is problematic; convalescing cells frequently have nutrient and incubation requirements different from those of unstressed cells (e.g., Pflug and Schmidt, In Sterilization, Disinfection, and Preservation, 1968). Generally the physiological state, as well as the species of the microorganisms in the population, must be known in order to design an effective test method.

Studies (e.g., Bond et al., 1970: Appl. Micro 20:573; Puleo et al., 1975: Appl. Micro 30:786) indicate that microorganisms recovered from the environment without alteration of their physiological state (e.g., no subculturing) are among the most resistant to lethal agents. Therefore, isolated, naturally occurring microbial populations are more appropriate for use in the evaluation of sterilants. In addition, it is desirable to consider the incorporation of standardized, pure cultured biological indicators in any sterility test protocol (e.g., Bruch, In Develop. Industrial Micro, 1973). It has also been demonstrated that certain naturally occurring isolates retain a high resistivity upon subculture; these so called "hardy" cultures make rigorous indicators.

9.2.2 Significant Accomplishments

Efforts during the present reporting period have been directed towards the development of a protocol for studying dry heat and gamma radiation as soil sterilants. Work has centered on methods to acquire, and use as biological indicators, naturally occurring microbes resident in soil.

9.2.2.1 Selection of a Recovery Medium. 5 microbial growth media were tested to determine maximum recovery efficiency of untreated (N_o) and heat-treated organisms. The following media were tested and results are given in Figure 9-B.1.

Trypticase Soy Agar (TSA:BBL)

Trypticase Soy Agar supplemented with 0.1% soluble starch and 0.2% Yeast Extract (TSAS:BBL)

NIH Agar (Difco)

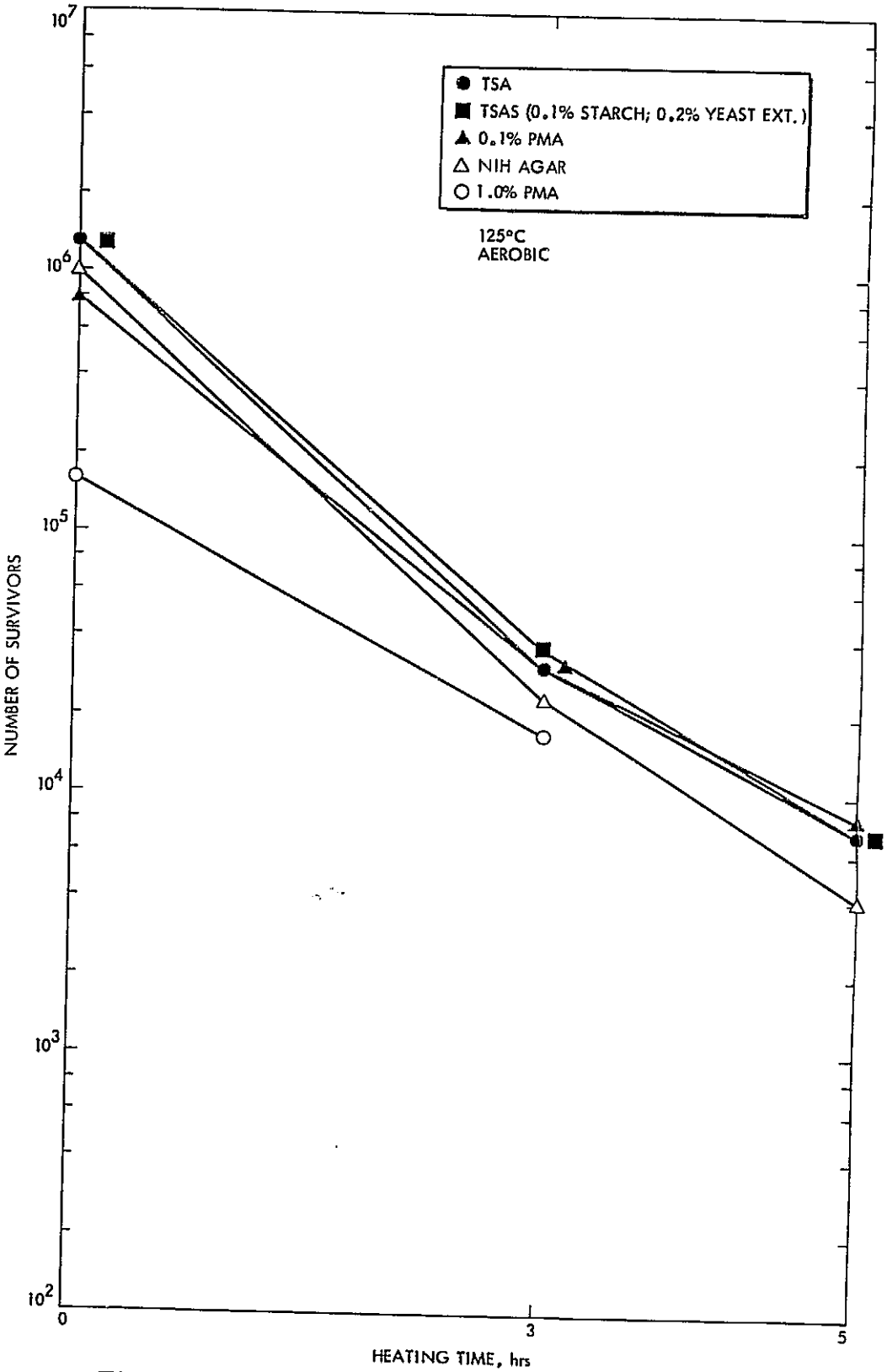


Fig. 9-B.1. Effect of selected nutrient medium on enumeration of soil organisms

0.1% Peptonized Milk Agar (0.1% PMA:BBL)

1.0% Peptonized Milk Agar (1.0% PMA:BBL)

It can be seen that TSA and TSAS were comparable in recovery efficiency while 0.1% PMA evidenced a lower recovery efficiency initially but proved equal to TSA and TSAS at the 3 and 5 hour heating interval with prolonged incubation (32°C, 7 days). Compared to 0.1% PMA, colonial development was much more rapid and larger using TSA and TSAS. Coupled with the results of this study, the extensive use of TSA by other investigators in this area led to its selection as the recovery medium for subsequent work.

9.2.2.2 Delineation of Survivor Curves. Soil was collected from the grounds of JPL and adjacent surroundings in sufficient quantity to perform several analyses. The soil was evenly spread on aluminum trays and dried at 50°C in a forced-draft dry heat (FDDH) oven for 24 hours, then gently ground with a mortar and pestle, and finally passed through a 2 mm sieve and collected into a single container.

10 gram aliquots were introduced into sterile 25 x 200 mm screw-capped test tubes for heating. 3 replicate tubes of soil were tested per heating interval, and N_o population. Type "T" copper constantan thermocouples (TC) were placed into 3 such tubes to monitor the heating cycle, and were recorded on a Honeywell Strip Chart recorder. A FDDH oven set at 125°C was utilized throughout. Timing of the dry heat cycle was initiated once the coldest TC reached 124°C and was maintained at 125 $\pm$ 1°C for the appropriate interval.

Following heating, the tubes were removed from the oven and allowed to cool on a laminar air flow clean bench. 30 ml of sterile distilled water was introduced into each tube and thoroughly mixed by vortex agitation for not less than (NLT) 1 minute. Each tube was then insonated at 25 KHz for 15 minutes in an ultrasonic waterbath, and again vortexed for NLT 1 minute. Heavy sediment was allowed to settle for 1 minute before performing 10-fold serial dilutions in sterile distilled water dilution blanks. Appropriate dilutions were plated with molten agar (45°C) and allowed to solidify. Each plate was then overlayered with molten 2% Agar. Following initial plating, the dilution blanks were heated at 80°C for 20 minutes in a circulating waterbath, and plated as above. All plates were incubated at 32°C for NLT 72 hours and colony forming units enumerated with the aid of a colony counter.

For anaerobic analyses additional plates were introduced into an anaerobic vessel, i.e., Gas Pak (BBL) then incubated and enumerated as above.

JPL soils were subjected to dry heat at 125°C for various time intervals to determine the point of non-linearity when the logarithm of the survivors was plotted against time at temperature.

It can be seen from Figures 9-B.2, 9-B.3, and 9-B.4 that heat shocked aerobic and anaerobic survivor curves demonstrate similar configurations. Figure 9-B.5 is a summary of all aerobic determinations performed.

By inspection of the curves it can be seen that a break occurs, and "tailing" of the survivor curves begins after approximately 5 hours heating. A 5 hour sublethal cycle was thus chosen to eliminate more susceptible subpopulations of organisms, to minimize the presence of heat damaged spores, and yield a naturally occurring dry heat resistant population.

It is the further objective of this effort to select and isolate (in sufficient numbers) organisms responsible for the tail portion of the survivor curve, i.e., those organisms surviving 5 hours of dry heat exposure, test them for retention of dry heat resistance, and use them as biological indicators in further studies.

9.2.3 Future Activities

Efforts will continue towards development of a naturally occurring soil population sterilization indicator. Dry heat and gamma radiation will be studied as soil sterilants under appropriate simulated conditions. Techniques will be developed to assess the amount of resident bioinformation remaining after exposure of soil to the sterilants.

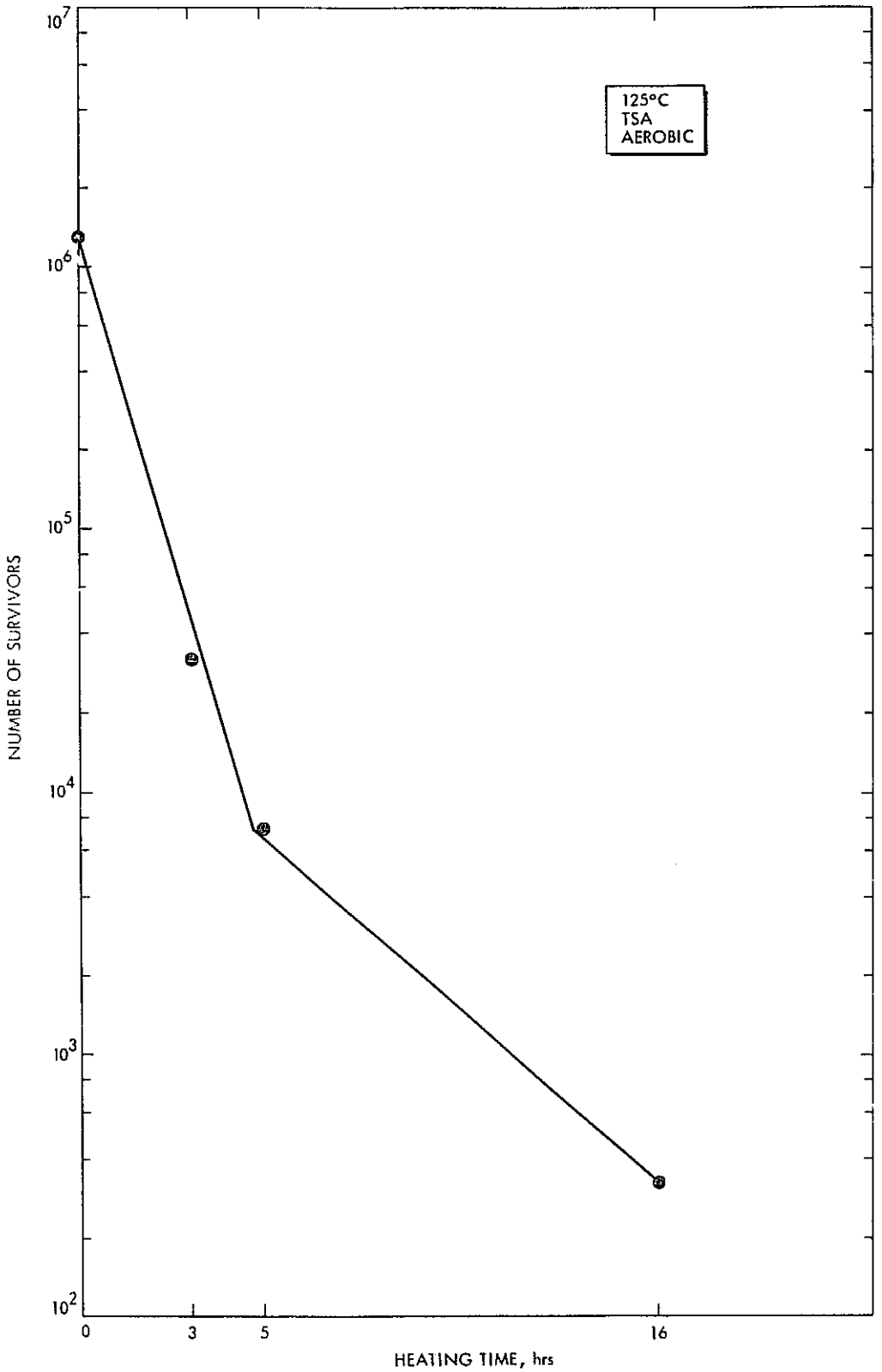


Fig. 9-B.2. Survival curve for aerobic soil organisms

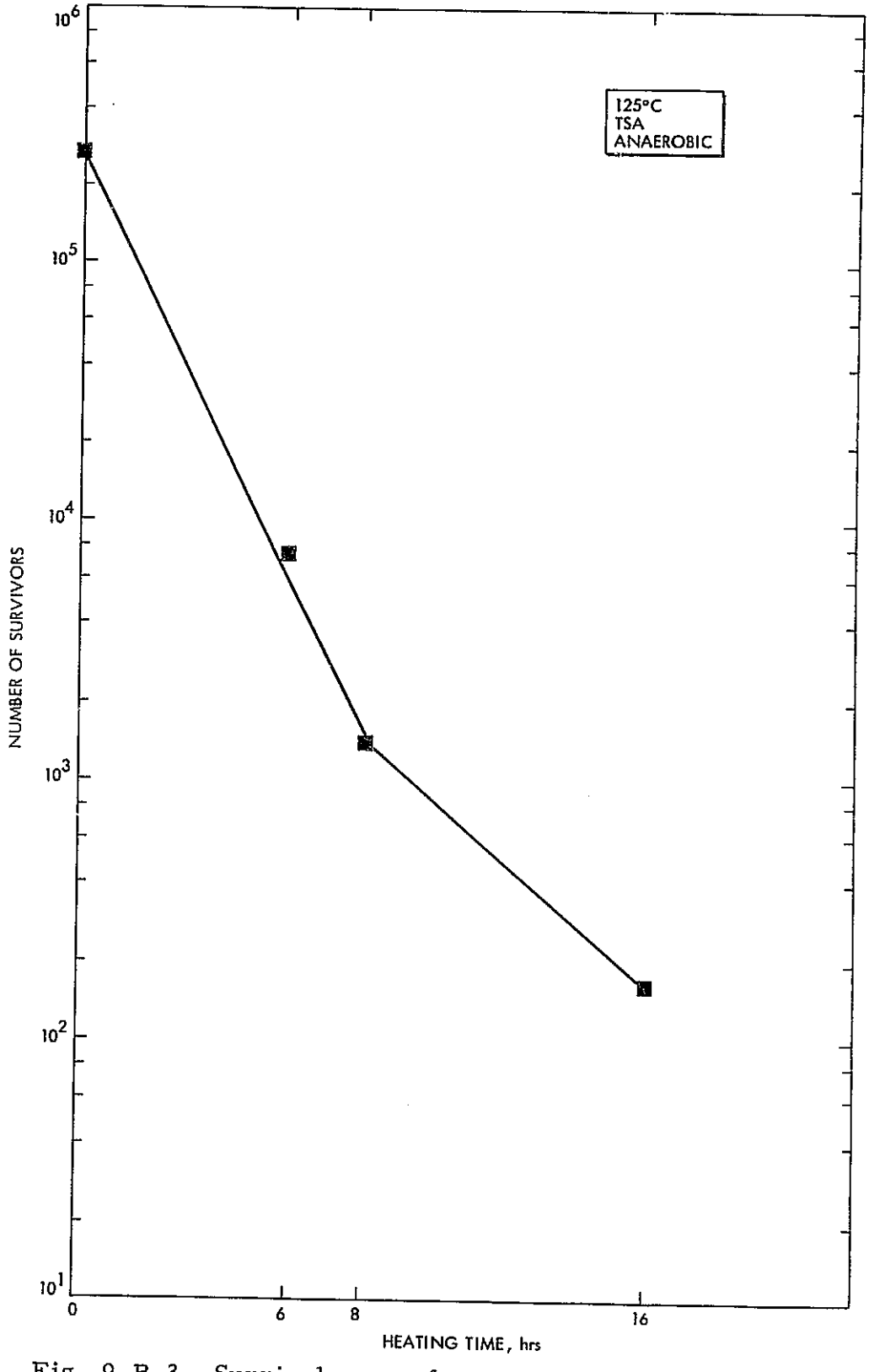


Fig. 9-B.3. Survival curve for anaerobic soil organisms

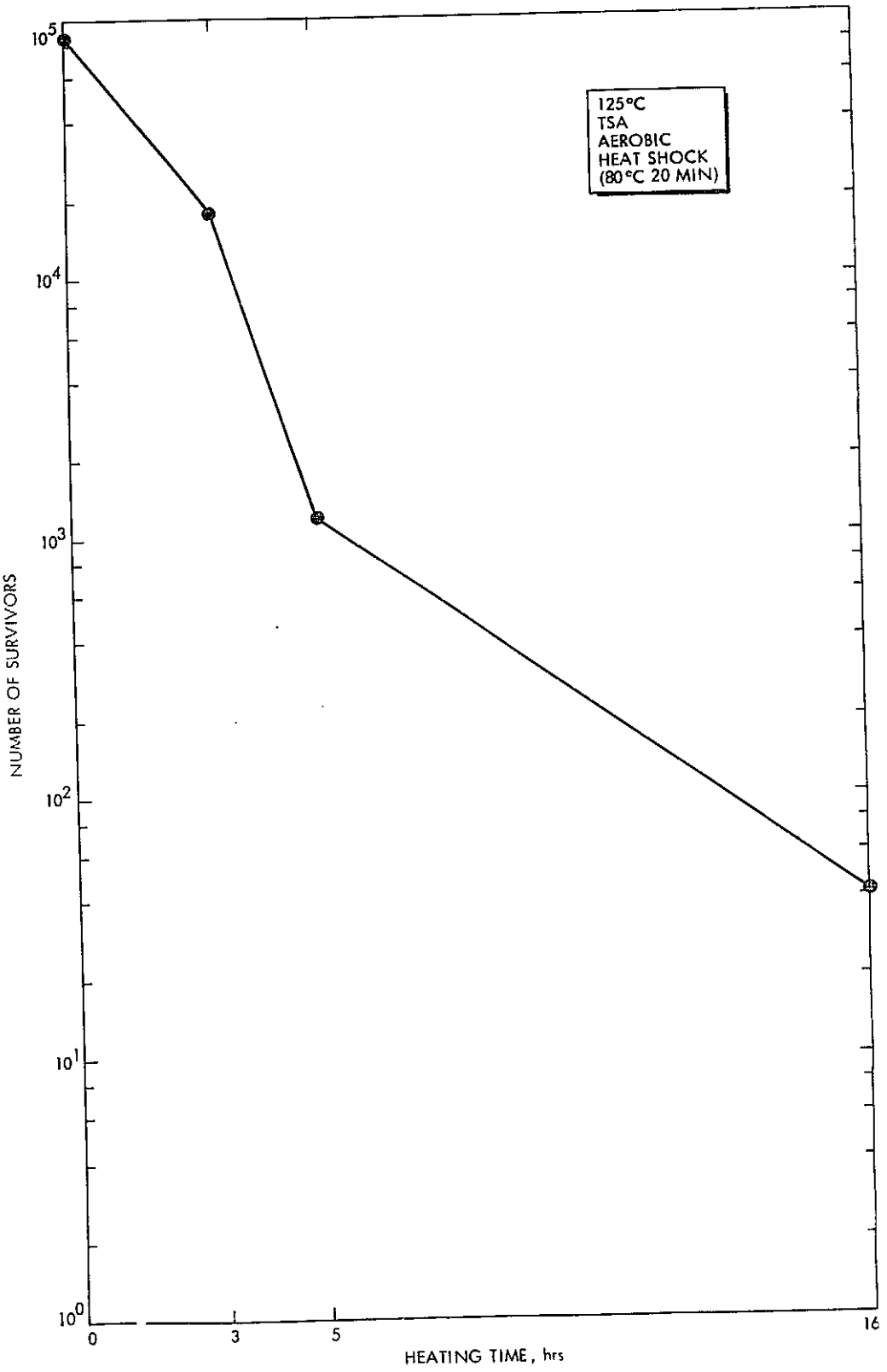


Fig. 9-B.4. Survival curve for heat shocked aerobes from soil

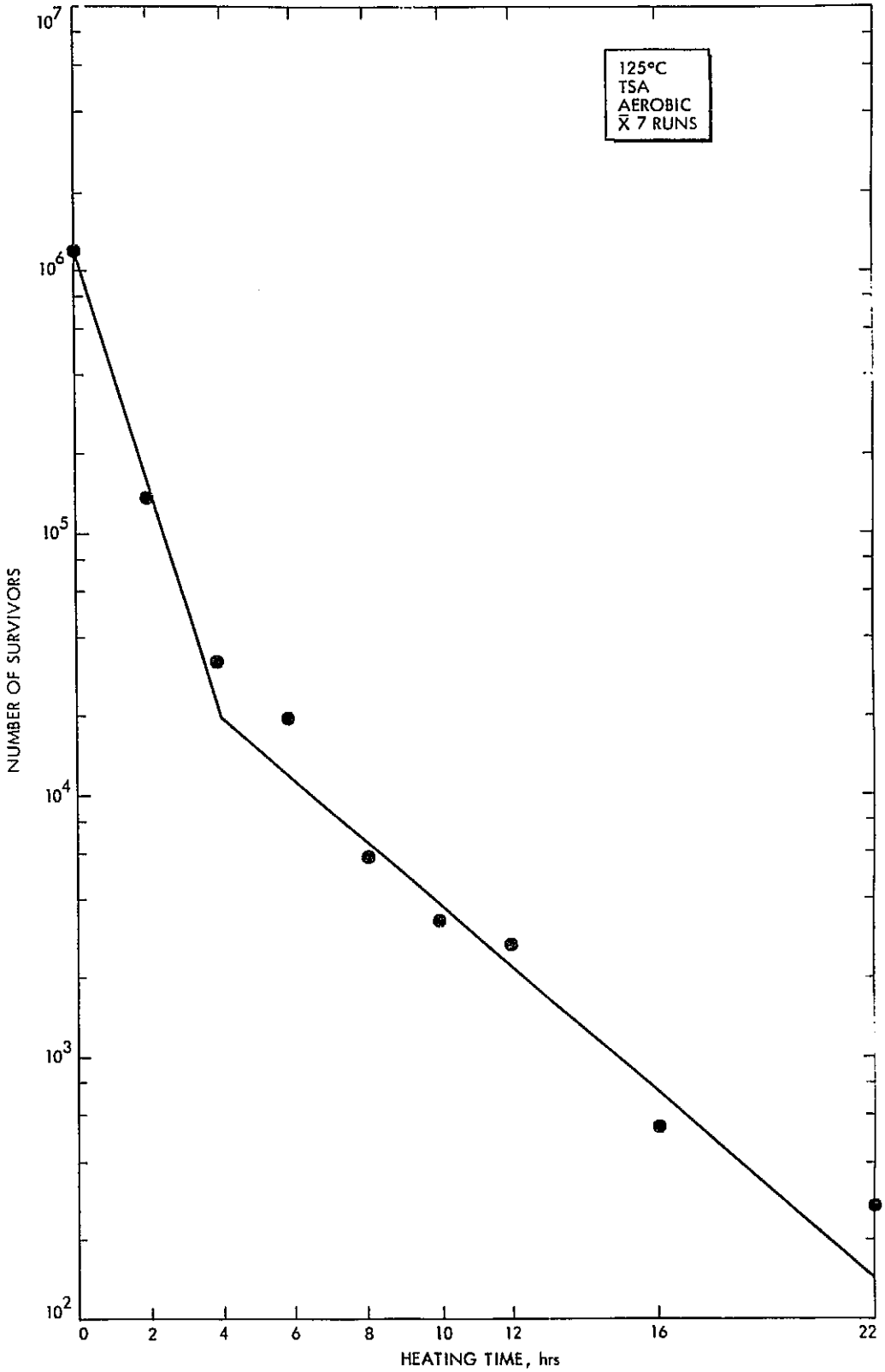


Fig. 9-B.5. Summary for aerobic survival tests for soil organisms

SECTION X

PLANETARY QUARANTINE LABORATORY ACTIVITIES (AFETR) NASA No. 195-58-63-06)

Contents	Title and Related Personnel
Subtask A para. 1.1	Planetary Quarantine Laboratory Assay Activities (AFETR)
	Cognizance: J. R. Puleo
	Associate Personnel: N. Fields, S. Bergstrom, G. Oxborrow, L. Maull, S. Rood (Bionetics)
Subtask B para. 1.2	Teflon Ribbon Experiments
	Cognizance: J. R. Puleo
	Associate Personnel: S. Bergstrom, G. Oxborrow
Subtask C para. 1.3	Pyrolysis Gas-Liquid Chromatography Study
	Cognizance: N. Fields
	Associate Personnel: G. Oxborrow, J. R. Puleo

SECTION X

PLANETARY QUARANTINE LABORATORY ASSAY ACTIVITIES (AFETR)

10.1 PLANETARY QUARANTINE LABORATORY ASSAY ACTIVITIES

10.1.1 Subtask A Introduction

The objective of this subtask is to determine and document the quantitative and qualitative microbiological profiles of pertinent automated outbound spacecraft which may carry terrestrial organisms to the planets.

This work is to implement current NASA policy and lays a foundation upon which future policy can be formulated.

10.1.2 Significant Accomplishments

Bioassays were completed on the Viking spacecraft. The results of the microbiological assessment of the spacecraft have been reported in Jet Propulsion Laboratory (JPL) Doc. 900-732, April 18, 1976. The Planetary Quarantine Laboratory (PQL) will continue to microbiologically assay spacecraft and spacecraft environments before, during and after final assembly, in order to develop a population profile of the microorganisms associated with the spacecraft and spacecraft assembly facilities. Plans are being formulated for the bioassay of the Mariner Jupiter/Saturn '77 spacecraft during final assembly and pre-launch operations at Cape Canaveral, Florida. Other pertinent automated outbound spacecraft will also be assayed, as such missions/spacecraft are authorized and as they each move through their pre-launch operations phase.

Sampling and assay methods currently used today are defined in NHB 5340.1A, "NASA Standard Procedures for the Microbial Examination of Space Hardware" (1968). These procedures were designed primarily for the detection and enumeration of aerobic, heterotrophic, mesophilic microorganisms recovered from surface areas and are, at this point in time, in need of updating. Over the past several years much experience has been gained in new sampling and microbiological assay techniques applicable to spacecraft and spacecraft assembly environments. Sampling techniques, which can be used to rapidly sample large surface areas, and assay methods which can incorporate

the recovery and enumeration of a broader spectrum of spacecraft microbial contaminants such as, psychrophilic and anaerobic microorganisms are needed.

Microbiological sampling of spacecraft surfaces has been restricted to the use of the swab-rinse technique. The cotton swab method, although adopted as standard procedure by the American Public Health Association, has several disadvantages. The surface area that can be sampled by a swab is small, there is poor correlation among investigators as to the amount of microbial contamination recovered. This is partially because different types of soil or dust to which microorganisms adhere are removed at different rates, depending on the chemical or physical properties of the surface. The release of microorganisms enmeshed in the cotton has been limited, causing reduced counts. Because of these and other undesirable properties that are inherent in this method, alternate techniques have been sought for the purpose of improving the reliability of surface sampling.

Studies have been initiated in the PQL directed towards developing a swab-rinse technique whereby large areas of spacecraft surfaces can be assayed. Two commercially available cellulose sponges were compared to cotton swabs. The results showed that the recovery efficiency of the cotton swab was 3-5 times greater than that observed for the sponge. Preliminary studies using a clean room fabric has shown that the recovery efficiency is 2-4 times greater than the cotton swabs. Studies are in process to evaluate five types of clean room materials.

10.1.2 Future Activities

Assessment of the contamination, both viable and non-viable, associated with various elements of the Space Transportation System (STS) will be initiated. Since it is planned that the STS will be used to launch all post 1980 planetary spacecraft, it is important to gain an understanding of the contamination background or profile. The difficulty in developing this understanding arises from the fact that the STS represents an entirely new situation or condition insofar as contamination is concerned. Hardware of a new design is involved, new organizational elements must be dealt with, new facilities have

been built, the launch and orbital environments are different, the launch vehicles are now reusable (presenting concerns relative to prior use); these and other factors combine to pose serious problems in the conduct of assay operations. Preliminary bioassays of those STS facilities which are used to assembly or process planetary spacecraft will be conducted as these facilities are completed.

Additionally, as early versions of the Shuttle Orbiter and Spacelab become available, and as accessibility conditions permit, bioassays will be conducted so as to begin the development of biological background profiles for these vehicles.

A prerequisite to the conduct of either spacecraft or facility assays is the development of appropriate assay plans, procedures and techniques. It is proposed to preliminarily develop such plans and methodology with specific applicability to the STS. This effort is not expected to pursue the development of new basic microbiological assay techniques, but rather to involve the modification or adaptation of existing techniques so as to be applicable to STS. Preliminary documentation of the "STS procedures" is proposed to be generated which can be used to start initial technical dialogue with those responsible for other aspects of STS development.

In summary, future work will be directed to the microbiological assessment of spacecraft and spacecraft environments before, during, and after final assembly, in order to develop a population profile of the microorganisms associated with the spacecraft and spacecraft assembly facilities. Bioassay of pertinent automated outbound spacecraft such as Mariner Jupiter/Saturn '77, will be performed. Microbiological assessment of the various elements of the STS System will be initiated. Bioassays will be performed on the Payload Bay and Spacelab Module of initial flights of the Space Transportation System (STS) to establish a biological background. This will include a quantitative and qualitative microbial assessment of the intramural environment of the STS facilities. Research into new sampling and microbiological assay techniques applicable to spacecraft and spacecraft assembly environments will be continued.

10.1.3 Presentations

Puleo, J. R., N. D. Fields, S. L. Bergstrom, and G. S. Oxborrow. Viking Bioassay Activities. Presented at the Committee on Space Research (COSPAR), Philadelphia, Pennsylvania, June 14-17, 1976.

10.1.4 Publications

Puleo, J. R., N. D. Fields, S. L. Bergstrom, and G. S. Oxborrow. Microbiological Profiles of Viking Spacecraft. Submitted for publication in Applied and Environmental Microbiology, July, 1976.

10.2 TEFLON RIBBON EXPERIMENTS

10.2.1 Subtask B Introduction

The objective of this study is to characterize the thermal resistance profiles of naturally occurring bacterial spores associated with assembly facilities at Kennedy Space Center.

The validity of the currently accepted sterilization cycle should be confirmed. The cycle, to be valid, should be effective on bacterial spores associated with spacecraft in residence at Kennedy Space Center, Florida.

10.2.2 Significant Accomplishments

The teflon ribbon study was resumed in January 1976. This study was discontinued in May 1975 so as to increase the effort being applied to the Viking '75 Program. The details of the thermal apparatus and experimental test procedures were described in para. 5.1.2.1 of Jet Propulsion Laboratory (JPL) Doc. No. 900-655, April, 1974. Naturally occurring airborne bacterial spores were collected on teflon ribbons exposed to the intramural environment of the Vehicle Assembly Building (VAB), Kennedy Space Center (KSC), Florida.

In any dry heat sterilization cycle, there are several factors which may effect the efficiency of the process. One of these parameters is the effect of temperature. The thermal resistance of a mixed naturally occurring spore population from the VAB has been extensively examined in the temperature

range around 111.7C. Work with a pure culture of B. subtilis var. niger (B. globigii) spores led the investigators to the following hypothesis.

The hypothesis proposed states that a reduction in the "Survival Fraction" of the "hardy" organisms will occur as the temperature is increased, provided that all other factors remain constant. This reduction will follow an initial slope similar to that shown in Figure 10-B.1 and this slope will then level off and remain relatively constant over an extended temperature range before again falling off. An experiment was designed to examine this hypothesis. The objective was to estimate the reduction in a mixed naturally occurring spore population at six temperatures using the Viking thermal cycle (30 hours at temperature) and a water concentration of 1.2 mg/L.

The results of the thermal experiments are shown in Tables 10.B.1 and 10.B.2. The results show that as the temperature is increased from 111.7C to 135C, the "Survival Fraction" is decreased from a mean of 1.3×10^{-3} to a mean of 3.4×10^{-5}. An initial reduction is observed between 111.7C and 120C with a leveling off after 120C. Preliminary results of the

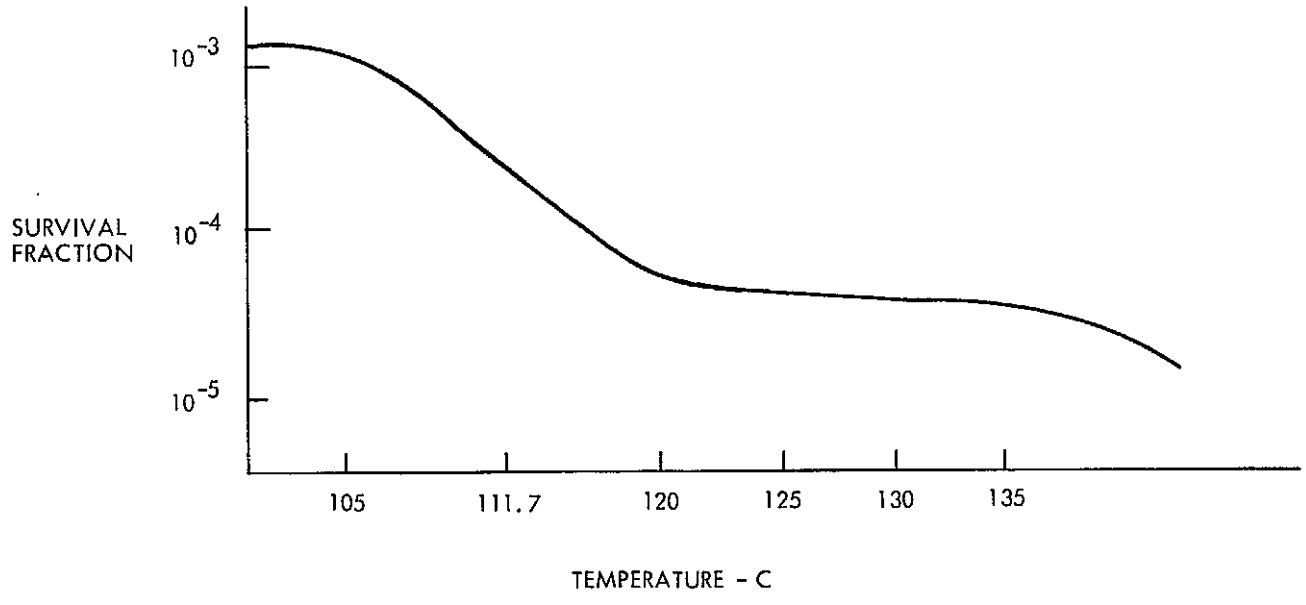


Fig. 10-B.1. Effect of temperature on survival fraction

Table 10-B.1. Thermal resistance of bacterial spores collected on teflon ribbons - VAC - KSC

30 Hour Cycle
1.2 mg/Liter Water

Experiment Number	N_o Spores[a]	Positive/ Total	MPN for Ribbon	Survivor Fraction[b] N_H/N_O
Temperature 111.7C				
10-27D	9.8×10^2	17/24	1.232	1.3×10^{-3}
11-27D	1.1×10^3	20/24	1.792	1.6×10^{-3}
12-27D	1.6×10^3	22/24	2.485	1.6×10^{-3}
13-27D	1.8×10^3	23/24	3.179	1.8×10^{-3}
14-27D	4.6×10^2	16/24	1.099	2.4×10^{-3}
15-27D	2.6×10^3	23/24	3.179	1.2×10^{-3}
Mean	1.4×10^3	121/144	1.834	1.3×10^{-3}
Temperature 120C				
16-27D	2.3×10^3	14/24	0.876	3.7×10^{-4}
17-27D	6.4×10^2	1/24	0.043	6.7×10^{-5}
18-27D	5.4×10^2	1/24	0.043	8.0×10^{-5}
19-27D	6.2×10^2	2/24	0.087	1.4×10^{-4}
20-27D	4.8×10^2	1/24	0.043	8.9×10^{-5}
21-27D	8.0×10^2	3/24	0.134	1.7×10^{-4}
Mean	9.0×10^2	22/144	0.165	1.8×10^{-4}

[a] N_o = Initial Spore Population

[b] N_H/N_O = Total number of "hardy" organisms N_H, surviving a thermal cycle, divided by total initial population N_O.

Table 10-B.2. Thermal resistance of bacterial spores collected on teflon ribbons - VAC - KSC

30 Hour Cycle
1.2 mg/Liter Water

Experiment Number	N_o Spores[a]	Positive/ Total	MPN for Ribbon	Survivor Fraction[b] N_H/N_O
Temperature 125C				
22-27D	2.9×10^3	4/24	0.182	6.2×10^{-5}
23-27D	2.5×10^3	2/24	0.087	3.5×10^{-5}
24-27D	1.2×10^3	1/24	0.043	3.5×10^{-5}
25-27D	1.8×10^3	1/24	0.043	2.4×10^{-5}
26-27D	1.8×10^3	3/24	0.134	7.3×10^{-5}
27-27D	1.5×10^3	1/24	0.043	2.8×10^{-5}
Mean	2.0×10^3	12/144	0.087	4.4×10^{-5}
Temperature 130C				
29-27D	1.2×10^3	3/24	0.134	1.1×10^{-4}
30-27D	6.3×10^2	0/24	0.0	0.0
31-27D	4.2×10^2	0/24	0.0	0.0
32-27D	3.3×10^2	2/24	0.087	2.7×10^{-4}
33-27D	3.9×10^2	1/24	0.043	1.1×10^{-4}
34-27D	2.5×10^2	0/24	0.0	0.0
Mean	5.4×10^2	6/144	0.043	7.8×10^{-5}
Temperature 135C				
35-27D	7.1×10^2	0/24	0.0	0.0
36-27D	5.5×10^2	1/24	0.043	7.8×10^{-5}
37-27D	3.5×10^2	1/24	0.043	1.2×10^{-4}
38-27D	6.3×10^2	0/24	0.0	0.0
39-27D	6.5×10^2	1/24	0.043	6.6×10^{-5}
40-27D	7.8×10^2	0/24	0.0	0.0
Mean	6.1×10^2	3/144	0.021	3.4×10^{-5}

[a] N_o = Initial Spore Population

[b] $N_H N_O$ = Total number of "hardy" organisms N_H, surviving a thermal cycle, divided by total initial population N_O.

105C experiments indicate that the hypothesis is correct. The effect of this lower temperature on the "Survivor Fraction" will be presented in the next report.

Those organisms which survived the high thermal inertia cycle were isolated and subcultured for identification. The results are recorded in Table 10-B.3. A total of 164 ribbons have shown signs of bacterial growth after addition of nutrients and incubation. Some difficulty was encountered in successively subculturing a number of isolates for inoculation into identification media. More than one attempt was made before growth on subculture was sufficient to obtain a suspension of high enough concentration to use as an inoculum. Difficulty in isolation appeared to be related to the temperature attained during the high thermal inertia cycle. One hundred and fifty-one isolates were identified throughout the course of the investigation.

As the temperature increased from 111.7C to 135C, a decrease in the number of species isolated was observed. An exception was noted in the experiments subjected to a temperature of 130C. Table 1-B.3 shows that Bacillus lentus and the atypical Bacillus group constituted the greater percentage of the heat survivors recovered. These data are consistent with those obtained in previous work (JPL Doc. No. 900-675, September 1974; JPL Doc. No. 900-701, April 1975; JPL Doc. No. 900-715, October 1975). The number of atypical Bacillus isolated accounted for as many as 86% of the total isolates. Organisms which exhibit a lack of biochemical activity are placed into this category, and may, therefore, not be identical either morphologically or genetically.

The majority of the isolates obtained from the heat studies were not identifiable to a particular species. Due to the small number of isolates obtained at temperatures above 111.7C, it was not possible to achieve a statistically valid view of the kinds and types of organisms which are recovered at the elevated temperatures. Bacillus brevis did occur with some regularity. This species exhibits two positive biochemical test reactions, casein hydrolysis and the utilization of tyrosine, but also has several variable test reactions. Theoretically, an identification to this species could be made on the basis of only six biochemical test reactions or growth capabilities. This is in contrast to at least eight reactions which must match exactly in order to make an identification to any other species.

Table 10-B.3. Types and levels of microorganisms recovered from teflon ribbons

Temp °C	Organism	No. Isolated	% Isolated
111.7	B. brevis	5	4.63
	B. circulans	7	6.48
	B. lentus	51	47.22
	B. sphaericus	1	0.93
	Atypical Bacillus	39	36.11
	Actinomycetes	5	4.63
120	B. lentus	2	9.52
	Atypical Bacillus	18	85.71
	Actinomycetes	1	4.76
125	B. brevis	2	18.18
	Atypical Bacillus	9	81.82
130	B. brevis	1	16.67
	B. lentus	1	16.67
	Atypical Bacillus	1	16.67
	Actinomycetes	3	50.00
135	B. brevis	1	33.33
	Atypical Bacillus	2	66.67

Note: Standard conditions of 1.2 mg/l moisture and 30 hours at temperature were maintained at a given temperature.

Studies were initiated to characterize the thermal resistant actinomycetes recovered from the teflon ribbon study. These organisms are bacterial in nature, form endospores, and are known to be very resistant. To establish the presence of actinomycetes as a constituent of the normal microbial fallout on teflon ribbons, various media and culturing techniques were used to isolate these organisms from the teflon ribbons used for determining the initial population. The use of a tap water and agar medium, Eugonagar, Trypticase Soy Agar, and a glucose-sodium nitrate medium did support the growth of fungi, but was not successful in recovering actinomycetes from the ribbons. All Actinomycetes recovered from the thermal experiments were isolated and stained for morphology. An acid-fast stain was also made to determine if any of the actinomycetes could be placed into a specific genus. No acid-fast cells were noted.

10.2.3 Future Activities

Future work will be directed to:

1) characterize the thermal resistance profiles of naturally occurring bacterial spores from assembly facilities at KSC.

2) develop thermal death curves using various temperatures, water concentrations and duration of thermal exposure.

3) develop and field evaluate assay techniques and procedures.

4) characterize the thermal resistant actinomycetes recovered from the teflon ribbon study, and determine their heat inactivation kinetics.

5) continue cooperative studies with Hardin-Simmons University to determine the occurrence of mesophilic and psychrophilic anaerobic microorganisms with unusual thermal resistance.

6) continue cooperative studies with the Food and Drug Administration to determine thermal inactivation of homogeneous and heterogenous bacterial spore populations.

10.3 PYROLYSIS GAS-LIQUID CHROMATOGRAPHY STUDIES

10.3.1 Subtask C Introduction

The objective of this investigation is to assess the usefulness of pyrolysis gas-liquid chromatography (PGLC) as a rapid reliable method of characterization or identification of members of the genus Bacillus.

The variability in the morphological and biochemical data obtained from the members of the genus Bacillus, particularly those encountered in the terminal decontamination studies, makes identification inconclusive. Forty to seventy percent of the isolates were identified as atypical after conventional tests had been applied. PGLC has been shown to give consistent identification of Salmonella, Mycobacterium and several other genera.

10.3.2 Significant Accomplishments

Preliminary results reported in para. 7.2.2 of Jet Propulsion Laboratory (JPL) Doc. No. 900-715, October, 1975, were verified in para. 8.2.2 of JPL Doc. 900-732, April, 1976. These results showed that if the membrane filter technique is used to grow bacterial cultures and if the media and culturing conditions are standardized, reproducible pyrochromatograms could be obtained for microorganism identification.

Recent publications have indicated that resolution of polar compounds could be enhanced by the use of a glass capillary column system. A study was initiated to evaluate a glass capillary system. Preliminary results using capillary columns prepared in our laboratory showed that much better resolution could be obtained using a glass capillary system. Difficulty in reproducing columns proved this method to be costly and time consuming. When a commercially prepared glass capillary column was available, it was purchased and the column tested for reproducibility in our laboratory. Advantages of using a commercially prepared column are: 1. Comparatively low cost; 2. Columns are graded by actual performance; 3. Performance is guaranteed (in terms of effective plate number); and 4. Columns would be standardized and available to other laboratories wishing to use our techniques.

A glass support coated open tubular column (SCOT) 0.5 mm I.D. x 25 Meter coated with Carbowax 20 M (Scientific Glass Engineering Pty. Ltd., Australia; GSB/CW20M/U/UM) was compared to the previously used 3.18 mm (1/8 inch) x 6 Meter glass column packed with 7% Carbowax 20M TPA on Anikrom ABS 100-110 mesh and was found to give much better peak separations. The glass SCOT column separated 140-145 chromatographic peaks compared to 60-64 on the 3.18 mm packed glass column (Figure 10-C.1). Figure 10-C.2 shows the pyrochromatograms of three different Bacillus spp. run on the glass SCOT capillary system. Differences between the species can be detected by a careful study of those areas between retention times of 0-5 and 20-40 minutes, resulting in a more efficient, economical, and reproducible system.

10.3.3 Future Activities

Future activities will be directed to:

1) continue pyrolysis of known bacterial cultures and environmental microbial isolates to complete a chromatographic catalogue;

2) determine correlation between physiological characteristics and peak variation;

3) identify or characterize dry-heat resistant microorganisms;

4) assess the effect of culturing techniques;

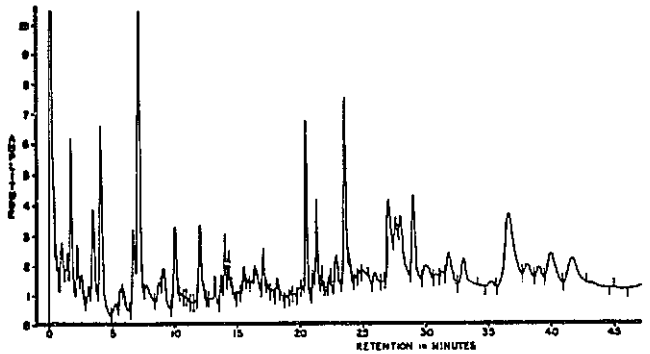
5) evaluate a pattern recognition computer identification system;

6) chemically identify resultant chromatographic peaks using mass spectroscopy; and

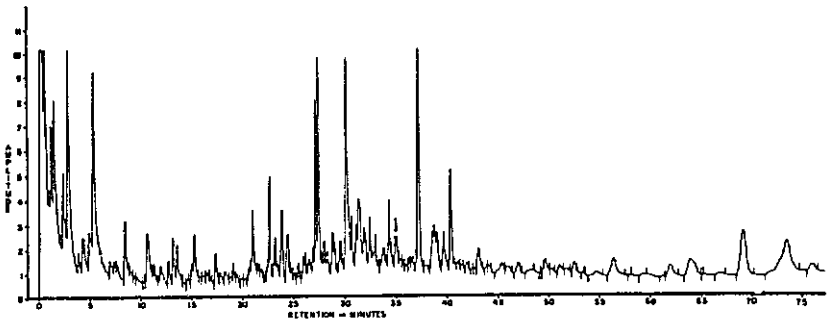
7) continue cooperative studies with Hardin-Simmons University for characterizing mesophilic and psychrophilic anaerobic microorganisms.

10.3.4 Presentations

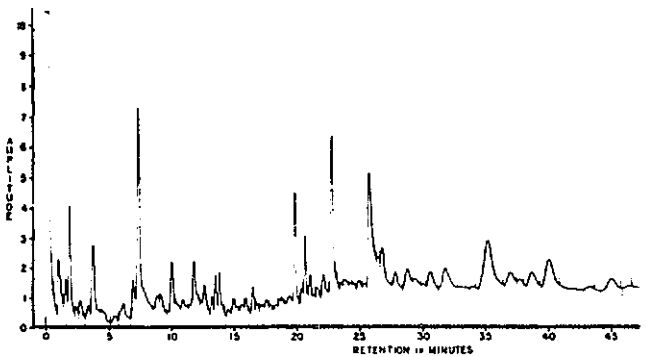
Oxborrow, G. S., N. D. Fields, and J. R. Puleo. Pyrolysis Gas-Liquid Chromatography Studies of the Genus Bacillus. Effect of Growth Media on Pyrochromatogram Reproducibility. Presented at the 76th Annual Meeting of the American Society for Microbiology, Atlantic City, New Jersey, May 2-7, 1976. ASM Abstracts, 1976.



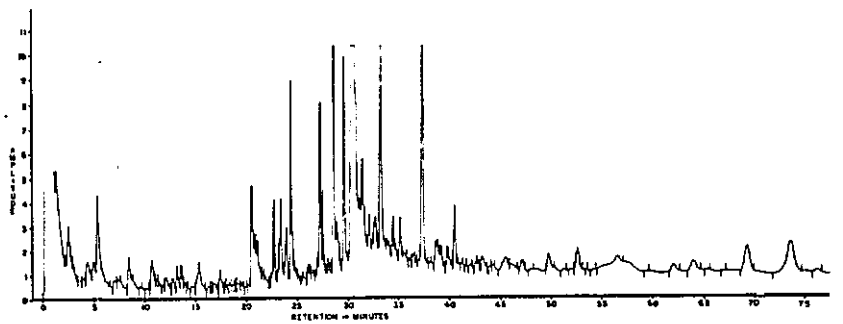
B. GLOBIGII 3.18 mm (1/8") PACKED GLASS COLUMN



B. GLOBIGII 1.59 mm (1/16") GLASS SCOT COLUMN

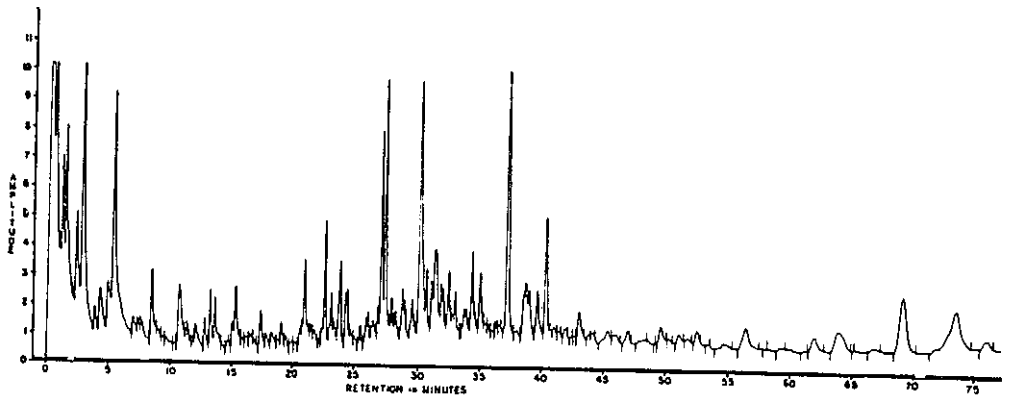


B. CEREUS 3.18 mm (1/8") PACKED GLASS COLUMN

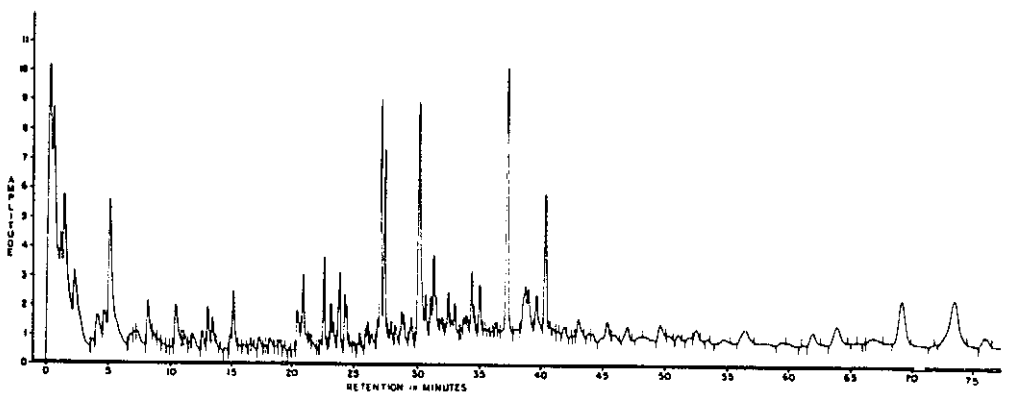


B. CEREUS 1.59 mm (1/16") GLASS SCOT COLUMN

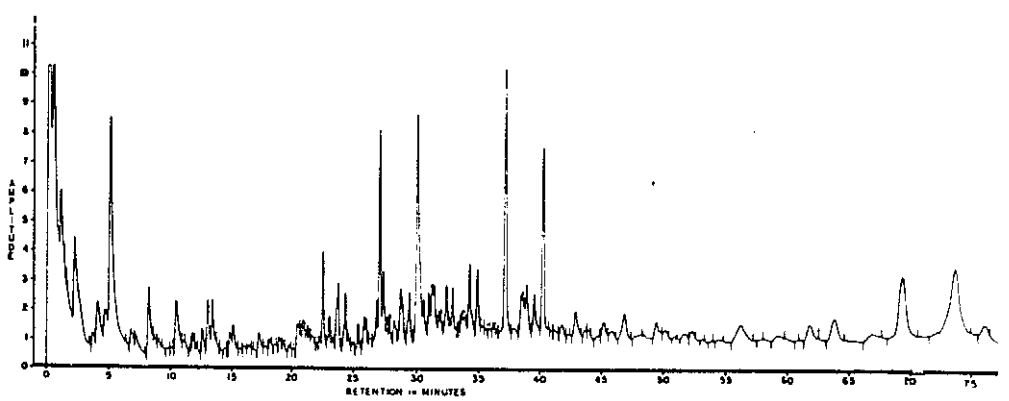
Fig. 10-C.1. Comparison of two different type glass columns



B. GLOBIGII 24 hr. @ 32 C.



B. COAGULANS #11369 24 hr. @ 32 C.



B. SPHAERICUS #7055 24 hr. @ 32 C.

Fig. 10-C.2. Pyrochromatograms of three Bacillus spp. using a glass SCOT capillary column system

10.3.5 Publications

Oxborrow, G. S., N. D. Fields, and J. R. Puleo. Preparation of Pure Microbiological Samples for Pyrolysis Gas-Liquid Chromatography Studies. Applied and Environmental Microbiology, Vol. 32, No. 2, August, 1976.

Oxborrow, G. S., N. D. Fields and J. R. Puleo. Pyrolysis Gas-Liquid Chromatography Studies of the Genus Bacillus. Effect of Growth Time on Pyrochromatogram Reproducibility. To be published in Proceedings of the Third International Symposium on Analytical Pyrolysis, Amsterdam, The Netherlands, Sept. 7-9, 1976.

APPENDIX A

PLANETARY QUARANTINE EVALUATION OF PROBE MISSIONS

APPENDIX A
PLANETARY QUARANTINE EVALUATION OF OUTER PLANET PROBE MISSIONS

A1. INTRODUCTION

The exploration of the outer planets by automated spacecraft began with the Pioneer Missions in 1973-74 and continue with the Mariner Missions to be launched in 1977. Since all of these missions were flybys and the liklihood of release and growth of terrestrial organisms is small, the Planetary Quarantine (PQ) constraints for these programs are readily satisfied with no spacecraft, ground operations, or trajectory penalties. For Jovian orbiters, the PQ constraints may not be as easily satisfied because of orbital lifetime considerations. For outer planet probes, the necessity of, as well as the degree of, sterilization/decontamination for satisfying the quarantine constraints are not understood. In order to do a realistic quarantine appraisal of a probe mission, a specific mission, outer planets orbiter/probe (Jupiter) - also referred to as Jupiter Orbiter Probe (JOP) - was selected for this investigation.

A1.1 JOP Mission and Spacecraft Description

A Mission utilizing an outer planet spacecraft is under consideration for a single launch during the 1981/1982 opportunity to delivery an entry probe in April 1985, and remain active in orbit around the planet for approximately one year. This proposed mission, designated the Outer Planet Orbiter/Probe (Jupiter) OPO/P(J) will offer the first opportunity to make <u>in situ</u> measurements of the Jovian atmosphere. It will also provide the opportunity to conduct extensive surveys of the planet, its environment, and its satellites utilizing orbits with apsides and orientations which are varied during the mission duration.

The Orbiter/Probe combination (i.e., the spacecraft) will be launched by a solid intermediate upper stage from a Shuttle. The spacecraft will be either a spin-stabilized (Pioneer type) or three-axis stabilized (Mariner type) spacecraft (reference 1). Flight time from spacecraft injection to Probe separation is approximately thirty-seven months. The spacecraft will be aimed directly at the planet with Probe separation occurring 56 days prior to JOI. At this time, the Orbiter will be deflected from its impact trajectory. The Orbiter will remain in orbit with a perijove of 3.0 R_j with an inclination of 15 degrees. The Probe will be delivered at a 7.5 degree entry angle at 6 degrees south latitude.

The Probe is released at a distance of 500 R_j* from Jupiter prior to performance of a maneuver to deflect the Orbiter vehicle from a planetary impact trajectory. The Probe is nominally targeted to a 7.8 degree (equatorial North) latitude, posigrade entry point with an inertial entry path angle

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*1 R_j = Distance equal to radius of Jupiter.

of -7.5 degrees. The Probe enters the atmosphere at a total angle of attack which is dependent upon the spacecraft/Probe separation conditions.

The characteristics of the Probe's motion will be determined by the descent trajectory and aerodynamic properties upon entry into the planet's sensible atmosphere. The entry flight path angle of -7.5 degrees limits entry deceleration to the 300 g_E level, which is well within the design value.

The following is a brief description of the Probe mission time-line for atmospheric descent as presented in Figure A1. The Probe enters the sensible atmosphere 56 days after separation from the spacecraft and encounters the atmospheric heating and deceleration environments. At a subsonic speed (M = 0.9) the in-situ measurement sequence is initiated by deploying the temperature sensor and mass spectrometer pressure inlet tube through the heat shield. The Probe continues to descend through the atmosphere relaying both stored and real time scientific data to Earth via the Orbiter. In-situ data is collected and transmitted to the spacecraft for the approximately 30 minute time span it takes to reach the 30 bar pressure level, wherein the communication levels geometry constraint is exceeded.

As for the Probe itself, it is a blunted 60 degree half-angle cone entry vehicle with a hemispherical afterbody, weighs 150 kg, and is 89 cm in diameter. A carbon phenolic heat shield covers the forward cone and remains on the Probe throughout atmospheric descent. The Probe spin rate at separation from the bus is nominally 5 rpm's. An external insulating blanket made of 25 layers of goldized mylar and nominally 1.21 cm thick covers the entire Probe. The Probe carries eight experiments which are provided to determine the physical structure and chemical composition of the upper portion of the troposphere, and the infrared radiation from the planet. Prior to the peak heat pulse, energetic particle and accelerometer data are gathered and stored for playback during real time data transmission.

While the Probe forebody is a single piece, carbon phenolic sphere, the after-body is a fiberglass-phenolic honeycomb hemisphere filled with a low density elastomeric ablation material. The forward ablator spherical nose section is 9.33 cm thick and the conical section is 5.39 cm thick. The carbon phenolic heat shield is bonded to a fiberglass face sheet 1.27 mm thick over a fiberglass-phenolic honeycomb core 2.15 cm thick. The honeycomb is in turn bonded by a high temperature adhesive to the outer surface of 1.52 mm thick aluminum cone (the primary structure). The honeycomb core is filled with powder. The heat shield is sized to dissipate the Jupiter entry heat load (primary by ablation) that is calculated for a Jupiter nominal atmosphere at an entry angle of -7.5 . A polyurethane foam cover forms the aft closure on the equipment.

The aft heat shield consists of a hemispherical fiberglass honeycomb sandwich 0.6 cm thick to which is bonded a 0.6 cm thick open honeycomb core. The core is filled with a low density elastomeric ablator by vacuum techniques.

The primary structure is designed for entry deceleration of 800 g_E with a safety factor of 1.25. The individual loads distributed over the structure are uniformly balanced by the atmospheric pressure loads that impinge on the heat shield face during entry, so that a minimum of bending is present

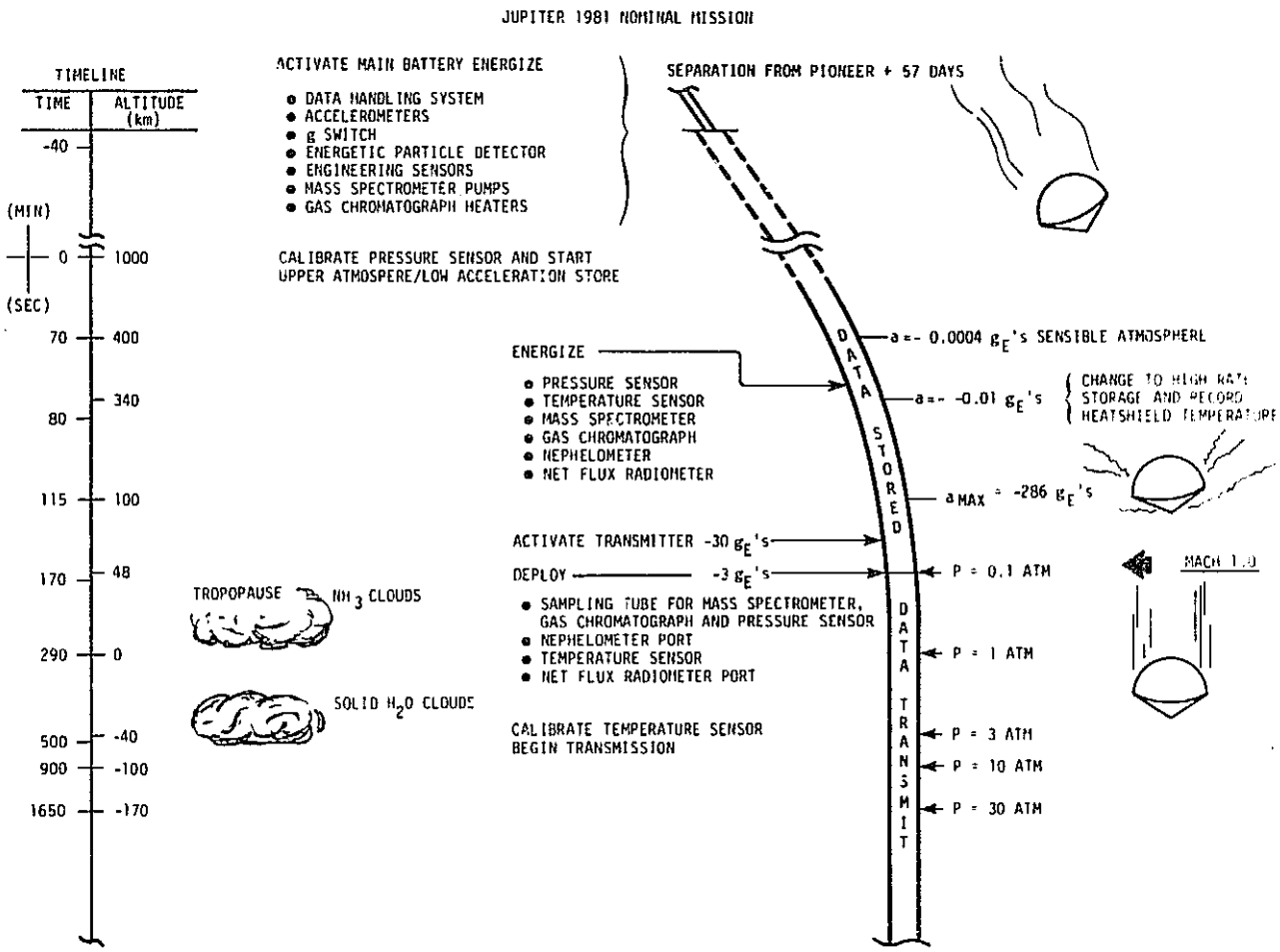

Figure A-1. Probe Time-Line for Jupiter 1981 Nominal Mission

on either the carbon phenolic heat shield or on the primary sandwich type structure. Controlled venting is permitted during Jovian descent so that pressure differentials remain small.

Probe release from the spacecraft is accomplished by simultaneous gas activation of three ball-lock release devices and use of three notched springs mounted concentrically with the release fittings.

Some of the science instrument sensors are extended through the heat shield after ejection of plug(s). Initiated by the appropriate accelerometer input, plug(s) is(are) ejected after maximum entry heating and at subsonic velocity.

A1.2 Prior Experience with Planetary Probes - The Viking Lander

In order to determine the allowable microbial burden on a planetary probe, the following equations are used:

$$P_C = NP_KP_RP_G \quad (1)$$

where P_C is the probability of contamination

where $$N = N_OP_{SL} \quad (2)$$

and N is the burden on the Probe at Launch.

N_O is the expected initial burden prior to sterilization.

P_{SL} is the probability that a single organism survives the sterilization exposure and is numerically equal to $10^{-t}/D_T$ where t is time.

D_T is the time it takes to cause a one log reduction by heating at temperature T.

P_K is the probability of microbial survival due to the space and planetary environments encountered.

P_R is the probability of release of a microorganism into the planetary environments encountered.

P_G is the probability of growth of a microorganism in the planetary environment, and is assumed equal to 1×10^{-7} for Jupiter, the current NASA guideline.

The procedure for determining the strategy for avoiding exceeding the probability of contamination allocation for a planet is based on demonstrating that the product of probabilities and N in equation (1) is equal to or less than P_C. The probability of release is based on the mode of release (such as breakoff, particle dislodgement, disintegration due to heating, etc.). The probability of kill is composed of the probabilities related to several environments and each must be determined separately. The results of determination of P_R and P_K, and assuming P_G equal to 10^{-7} determine the allowable value of N. N is turn determines the value of P_{SL} based on an estimate of N_O.

This report is concerned with the range of values to be expected for P_R and that part of P_K which occurs during the actual atmospheric entry. Reference will also be made to the experience with the Viking Lander in the achieving of the allowable value of N.

In the case of the Viking Lander (reference 2) the sterilization process proceeded in two stages. The first stage occurred as part of the flight acceptance testing (FAT) where components received dry heat exposure selected to reduce the encapsulated burden* by 2.07 decades, surface burden by 20.7 decades, surface burden by 20.7 decades, and mated burden by 10.35 decades. During this test the temperature of the case of most components rose to above 110°C. A second stage process, the terminal sterilization cycle, reduced the surface burden acquired after the first heating and that due to new mated surfaces. It also provided additional sterilization for encapsulated burden.

The basis for the initial estimate of the encapsulated burden taken from reference 3 is an estimate of 130 spores/cc of non-metallic material. The average density of 1.4 gm/cc was assumed for non-metallic material in order or derive the weight. The total heating experienced by the encapsulated burden was estimated to cause a reduction in burden of 8.5×10^{-3}.

Not all components were required to experience heating. FAT heating of the fibrous insulation used inside the Lander and on the aeroshell was waived because the material of which it is composed received an equivalent heat exposure during its manufacture. This possibility should also be considered in connection with materials used in an outer planet probe.

Another point to be considered is the use of filters in an air supply line. The use of N_2 to maintain a positive pressure in the Viking Lander caused a concern that microbial contamination would be introduced with the gas. Therefore, HEPA filters were used in the gas line. In one case three HEPA filters were used in series. Each has an efficiency of 99.97% in removing particles 0.3 microns or larger, providing a penetration probability of 2.7×10^{-11}.

A2. ANALYSIS AND TECHNICAL DISCUSSION

A2.1 Potential Contaminating Events

The PQ analysis model to be used in considering violation of a PQ constraint by an outer planet probe is an follows:

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*The three types of burden are defined to be:

Exposed - Burden on exposed surfaces.
Mated or Occluded - Burden on surfaces not exposed.
Encapsulated - Burden located in the interior of a material and entirely surrounded by it.

The probability of contamination for the planet P_c can be divided into two parts, $P_C(P)$ and $P_C(S/C)$ where $P_C(P)$ is the probability of contamination due to the probe and $P_C(S/C)$ is the probability of contamination due to the spacecraft or orbiter. Therefore,

$$P_C = P_C(p) + P_C(S/C) \tag{3}$$

A2.1.1 Probability of Contamination Due to Spacecraft.

The value of $P_C(S/C)$ is given by equation (4).

$$P_C(S/C) = NP_IP_GP_KP_R \tag{4}$$

where N, P_G and P_K were defined previously and P_I equals the probability that the spacecraft will impact the planet due to not being able to perform a successful separation with the probe and/or deflection maneuver to move it off its impact course.*

$$P_I = (1 - P_S)\ (1 - P_{D/\bar{S}}) + P_S\ (1 - P_{D/\bar{S}}) \tag{5}$$

where

P_S = probability of achieving a successful separation from probe.

$P_{D/\bar{S}}$ = probability of achieving a successful deflection maneuver given an unsuccessful separation maneuver.

$P_{D/S}$ = probability of achieving a successful deflection maneuver given a successful separation maneuver.

$$P_K = P_{SA}(P_{UV}XP_{VT})P_{SR} \tag{6}$$

P_{SA} = probability that an organism will survive the atmospheric entry heating

$(P_{UV}XP_{VT})$ = probability of an organism surviving the space environment.

P_{SR} = probability of an organism surviving Jupiter's trapped radiation belts.

P_R = Probability of an organism being released.

Values of the components of P_K and of P_R given in Table 1 are taken from reference 4.

The value of P_I can be determined from equation (5).

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*The probability of impact due to injection and midcourse maneuvers are not significant here since the final aim point has the spacecraft on an impact course with the planet.

A2.1.2 Probability of Contamination Due to the Probe.

The probability of contamination due to the probe can be broken up into a number of components.

$$P_C(P) = P_C(\overline{PN}) + P_C(PN) \tag{7}$$

where $P_C(\overline{PN})$ is the probability of contaminating the planet as a result of being unable to execute a nominal mission.

and $P_C(PN)$ is the probability of contaminating the planet during a nominal missio..

$$P_C\ (\overline{PN}) = P_C\ (\overline{PE}) + P_C\ (\overline{PV}) \tag{8}$$

where $P_C\ (\overline{PE})$ = is the probability of contamination due to being unable to enter the nominal entry corridor.

$P_C\ (\overline{PV})$ = is the probability of contamination due to probe break-up during a nominal mission due to a failure of the venting system.

$$P_C\ (PN) = P_C\ (PDeploy) + P_C\ (P\ Heats) + P_C\ (PV) + P_C\ (P\ Sens) + P_C\ (P\ Surv) \tag{9}$$

where P_C (PDeploy) is the probability of contaminating the planet as a result of a microorganisms released by extension of parachutes, deployable fins, etc., i.e., devices used to control the entry deceleration.

P_C (PHeatS) is the probability of contaminating planet due to micro-organisms released upon cracking of the heat shield.

P_C (PV) is the probability of contaminating the planet due to release of microorganisms through the probe venting system.

P_C (PSens) is the probability of contaminating the planet due to release of microorganisms through the probe instrument sensor ports.

P_C (PSurv) is the probability of contaminating the planet due to break-up of the probe after completion of nominal mission, but prior to, during, or soon after passage through the biozone.

A2.1.2.1 Contamination as a Result of Not Being Able to Execute a Nominal Mission. As given above, the equation giving the probability of contamination as a result of not being able to perform a nominal mission is

$$P_C\ (\overline{PN}) = P_C\ (\overline{PE}) + P_C\ (\overline{PV})$$

The probability of contamination due to non-nominal entry depends on the margin built into the structure and heat shield to withstand the excessive condition encountered by entering outside of the normal entry corridor. The steeper the entry, the greater the stresses encountered by the probe. The probability of probe break-up is a function of the departure from the

nominal corridor. Thus, if probability of departure from the entry corridor is represented by a probability density function.

$$P\ (\alpha)\ d\alpha$$

where α is angle of entry and the stress is given as a function of α as S (α).

Assume that the probe can withstand stresses less than S (α') where α' is the maximum entry angle for which the stress can be withstood by the probe.

The probability of probe break-up is then given by

$$\int_{\alpha'}^{90} P\ (\alpha)\ d\alpha \tag{10}$$

there

$$P_C\ (\overline{PE}) = \int_{\alpha'}^{90} P\ (\alpha)\ d\alpha \times P_R\ P_{SR} P_{SA} P_{SE} P_G{}^N \tag{11}$$

where P_R is the probability of release of a microorganism during break-up for release of a single or clump of organisms from the material on which they are enclosed or lie on

and P_{SR} is the probability that the organisms will survive the release

and P_{SA} is the probability of surviving atmospheric entry heating if not released prior to or during heating, if release takes place prior to or during heating, possible kill due to entry heating will appear in P_{SR}

and P_{SE} is the probability of surviving the space environment

and P_G is the probability of growth

and N is the potential number of organisms that may be released.

The probability of release P_R can be applied to surface, mated, and encapsulated burden. The following are based on values of P_R taken from reference 4: exposed or surface burden and mated burden, 1.0; encapsulated burden, 10^{-2}. For encapsulated burden release after late heating a P_R value of 10^{-3} is assumed. This lower value is assumed because plastic-like materials which contain encapsulated burden typically would be partially covered with a hard surface resulting from solidified material. In addition, the outer layers will have been brought to elevated temperatures.

The probability of surviving release is related to the probability of a single organism surviving release at the time of break-up and probe heating. In effect, it is the probability that an organism will survive the heating it receives upon release and is taken to be 10^{-4} for all burden types.

The probability of surviving the atmospheric entry heating is related to the heating received by organisms not released during break-up and probe heating. Therefore, this is heating received by an organism that remains with the probe and is released after the heat pulse is over. The value for exposed and mated burden taken from reference 4 are 10^{-4} and 10^{-3}, respectively; the value used for encapsulated burden is taken from reference 5 and represents a change from that value given in reference 4. This value is 10^{-1} and is used here.

The probability of surviving the space environment taken from reference 3 for Jupiter is 8×10^{-4} for exposed burden, 1.0 for mated burden, and 1.0 for encapsulated burden.

The probability of growth is 10^{-7}. A summary of the probability values is given in Table A1.

Table A1. Probability Values for Various Types of Burden

Burden Type	Probability	Early Release Values	Late Release Values
Exposed or Surface	P_R	1.0	1.0
	P_{SR}	10^{-4}	1.0
	P_{SA}	--	10^{-4}
	P_{SE}	8×10^{-4}	8×10^{-4}
	P_G	10^{-7}	10^{-7}
Mated and Interior Surface	P_R	1.0	1.0
	P_{SR}	10^{-4}	10^{-3}
	P_{SA}	--	10^{-1}
	P_{SE}	10^{-1}	10^{-7}
	P_G	10^{-7}	
Encapsulated Burden	P_R	10^{-2}	10^{-3}
	P_{SR}	10^{-4}	1.0
	P_{SA}	--	10^{-1}
	P_{SE}	1.0	1.0
	P_G	10^{-7}	10^{-7}

Estimates of N, the potential number of organisms that may be released, are based on the values given in Table A2 (reference 2) and comparison with the Viking Lander.

Table A2. Estimates of Spore Concentration

Number of spores encpasulated in material	130 spores/cm^3
Number of spores on surface	1.08 x 0^3 spores/m^2
Number of spores	1.5 x $^{\cdot}0^4$ spores/Kg

The total surface area of the probe assuming the model shown in Figure A2 is 1.95 m^2.

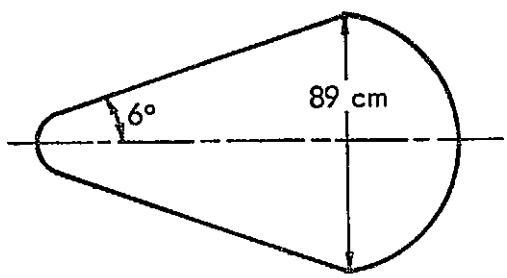

Fig. A2. Schematic of Probe Physical Configuration

Therefore, we could expect an exterior or interior surface burden of about 2.1 x 10^3 spores. The total volume of the probe using the above model is 2.4 x 10^5 cm^3 (approximate), with 5.3 x 10^4 cm^3 in the forebody which contains the instrumentation. The total volume of material which could contain encapsulated material on the Viking Lander (VL) was 6 x 10^4 cm^3. Therefore, considering the forebody volume of 5.3 x 10^4 cm^3, it would be expected that a probe contains less material with encapsulated burden than Viking. Assuming no more than .5 of the volume is devoted to encapsulated material, this volume is 2.5 x 10^4 cm^3. Therefore, the number of spores would be 3.25 x 10^6 compared to a Viking encapsulated burden of 8 x 10^6. This would appear to be a conservative estimate.

The instrumentation would also contain burden on the surfaces of the containers. The Viking Lander interior surface area is 105 m^2 which was estimated to contain 1.1 x 10^5 spores. The Viking Lander interior volume devoted to material containing encapsulated organisms is 6.0 x 10^4 cm^3 (approximate). It was assumed in the disqussion above that the corresponding quantity for the probe would be 2.5 x 10^4. Assuming the surface area would

be proportional to the total material contained, and using the encapsulated material as a guide, the ratio of the probe interior surface area to the Viking Lander interior surface would be .42. Therefore, assume the probe interior surface burden to be 4.6×10^4 spores. In terms of mated burden, assume the same ratio used for interior surface, i.e., .42. On the Viking Lander the mated surface area is 4.37 m^2 which gives an estimated mated surface area of about 1.8 m^2 for the probe. This would give a total number of spores of 2.4×10^3 spores for mated surface burden.

Table A3. Summary of Probe Burden Levels

Exposed or Surface	2.1×10^3
Mated Burden	2.4×10^3
Interior Surface	4.6×10^4
Encapsulated	3.3×10^6

Based on the values in Tables A1 and A3, the following values are obtained for the product of $P_R P_{SR} P_{SA} P_{SE} P_G N$:

Exposed or Surface Burden	early release	1.7×10^{-11}
	late release	1.7×10^{-11}
Mated Burden	early release	2.4×10^{-9}
	late release	2.4×10^{-8}
Interior Surface Burden	early release	4.6×10^{-8}
	late release	4.6×10^{-7}
Encapsulated Burden	early release	3.3×10^{-7}
	late release	3.3×10^{-5}

Combining these values gives 3.78×10^{-7} for early release (during peak heating) and 3.55×10^{-5} for late release. If a probability of contamination allocation for Jupiter close to 10^{-4} is assumed, a reasonable assumption for the amount allocated to $P_C(PE)$ would probably be no more than 0.1 that amount. Therefore, assume $P_C(PE)$ to be equal or less than $1. \times 10^{-5}$.

Therefore, if it is assumed that all of the organisms present are released either early or late, the maximum allowable value of

$$\int_{\alpha'}^{90} {}^{0}P(\alpha)\, d\alpha$$

is 1.0 or 0.3, respectively. The assumption that of all release occurs either early or late is too conservative, of course. If it is assumed that at least half of the burden is released early, the maximum allowable value is 0.6. This would give a minimum allowable probability of performing a successful entry of 0.4 represented by

$$\left\{1 - \int_{\alpha'}^{90} (P(\alpha)d\alpha\right\}.$$

This should be low enough to cause the problem of non-nominal entry to lose its significance. Any marginal change in the results given here would mean that some reduction of the en encpasulated burden would be necessary.

Consider the probability of contamination due to break-up after a nominal entry because of a malfunction of the venting system.

$$P_C\ (PV) = P_{vent}\ NP_RP_{SR}P_{SA}P_{SE}P_G \qquad (12)$$

where P_{vent} is the probability that the venting system will not work sa satisfactorily.

In this case the probability values can be separated again into those associated with early release and those associated with late release. The former values are the same as those in the similar case for non-nominal entry (Table A1). In the case of late entry there is one important difference. If break-up takes place after the heat pulse interior and encpasulated burden will not have received heat from the entry to cause any burden reduction. Table 4 summarizes the probability values for late release for venting failure.

In the case of break-up after peak heating, it must be assumed that no burden had been released prior to break-up. Therefore, the values of $P_RP_{SR}P_{SA}P_{SE}P_G{}^X$ N for each burden type are as follows:

Exposed surface burden	1.7×10^{-11}
Mated burden	2.4×10^{-5}
Interior burden	4.6×10^{-4}
Encapsulated burden	3.3×10^{-3}

Combining these values gives 3.78×10^{-3}. If we assume that the allocation for $P_C(PV)$ is about 1×10^{-5} then P_{vent} will have to be less than or equal to 3.8×10^{-3}; or the probability of successful venting operation will have to be greater than or equal to .997. The alternative is to reduce the encapsulated burden by three orders of magnitude.

Table A4. Probability Values for Various Types of Burden for the Case of a Venting Failure.

Burden Type	Probability	Value
Exposed on Surface	P_R	1.0
	P_{SR}	1.0
	P_{SA}	10^{-4}
	P_{SE}	8×10^{-4}
	P_G	10^{-7}
Mated and Interior	P_R	1.0
	P_{SR}	1.0
	P_{SA}	1.0
	P_{SE}	10^{-1}
	P_G	10^{-7}
Encapsulated	P_R	10^{-2}
	P_{SR}	1.0
	P_{SA}	1.0
	P_{SE}	1.0
	P_G	10^{-7}

A2.1.2.2 Contamination During Execution of a Nominal Mission.

1) Events Which Occur Prior to, or During, Peak Heating.

a) Extension of Parachutes, Deployable Fins, Other Aerodynamic Devices. The current probe description for JOP does not include parachutes, deployable fins, or other such aerodynamic devices. However, if such devices are used they will release organisms into the atmosphere before and after deployment.

In the case of the Viking Lander, it was estimated that the parachute contained an approximate volume of 2.8×10^4 cm^3 with an encapsulated burden of 3.6×10^6 organisms. This included the parachute mortar, parachute insulation assembly, as well as the parachute volume. The surface burden was estimated to be 1.3×10^3 organisms.

In the case of encapsulation burden the following equation will apply:

$$P_C\ (D(Enc)) = NP_R P_{SR} P_{SE} P_G \qquad (13)$$

where P_C (D(Enc)) is the probability of contamination due to the devices containing encapsulated burden and the rest of the parameters have the same definition given earlier.

Using the values given in Table 1 for early release, the value of right side of equation (13) becomes:

$N \times 10^{-13}$.

if we use a value of N of 3.6×10^6, $P_C(D(Enc))$ becomes 3.6×10^{-7}.

Applying the same equation and the values of early release from Table A-1 for surface burden gives:

$N \times (8 \times 10^{-15})$ or given that $N = 1.3 \times 10^3$

$P_C(D(S)) = 1.04 \times 10^{-11}$

where $P_C(D(S))$ is the probability of contamination due to devices containing surface burden.

Therefore, if a burden level equivalent to that estimated for the Viking Lander is assumed for release of organisms by parachutes and other devices prior to or during peak heating this contamination mode will not be a major problem.

b) Cracking of the Heat Shield. Some cracking of the heat shield during nominal entry is possible. This cracking could cause debris which is encapsulated in the heat shield to be released. In addition, for the probe design studies, there is a honeycomb structure behind the heat shield which is filled with powder. A crack in the heat shield could cause release of powder.

Figure A3 taken from Reference 1, gives the temperature profile of the heat shield and the honeycomb structure. If the assumption is made that this profile is a reasonably accurate representation of temperature in the heat shield then it can be considered to be sterile, or at least to contain a sufficiently small number of organisms so that contamination would not be a problem. In any case, equation 13 would represent this situation (with P_C for the heat shield), where $P_{SR} = 10^{-4}$, $P_{SE} = 1$ and $P_G = 10^{-7}$. Since the case of heat shield cracking is being considered, P_R can be assumed to be one (i.e., assume that cracking of the heat shield will lead to release of any organisms there). Therefore, the product $NP_R P_{SR} P_G$ becomes

N x 10^{-11}. Therefore, N could be 10^3-10^4 and still allow a reasonable margin of safety.

The powder in the honeycomb structure behind the heat shield could be a source of contamination. Using the Viking Lander, which has about 6.5 Kilograms of insulation with an estimated burden count of 10^5 organisms, as a guide and equation (13) with P_R = 1, $P_{SR} = 10^{-4}$, P_{SE} = 1 and $P_G = 10^7$ gives 10^{-6} for the probability of contamination. This is a marginal value and some burden reduction can be easily obtained by heating the powder prior to encapsulating it in the honeycomb. For example, heating it to 150 C would achieve about a two log reduction in burden in twenty minutes (reference 2).

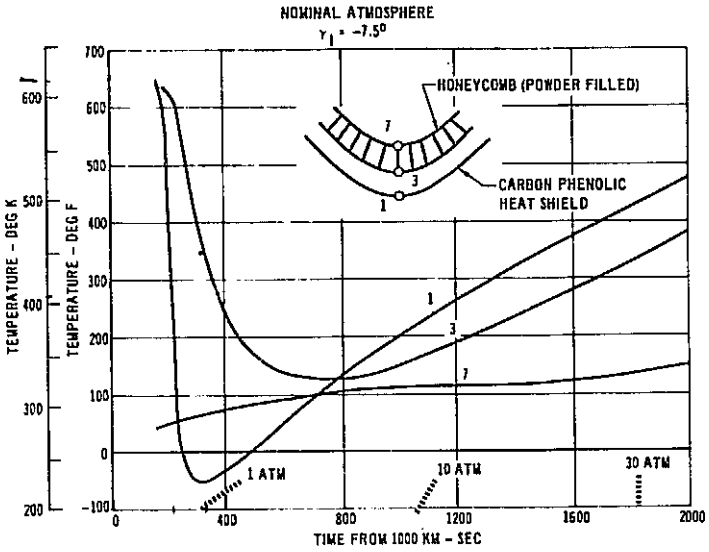

Figure A3. Forebody Heat Shield Temperatures

Another possible way of preventing contamination by this mode is to replace the 0.127 cm (.050 in) fiberglass facesheet separating the heat shield and honeycomb structure with something more substantial.

c) Release of Contamination Through the Probe Venting System. The probe will have a venting system which vents into the probe cavity in order to equilibrate the internal and external pressures. Although the purpose of the vents is to admit ambient atmospheric gas, if instabilities in gas flow occur due to internal pressure oscillation set up by the probe motion, especially spinning, internal particles may be released. Equation 13 describes the situation.

$$P_C(\text{Vent. release}) = N' P_R P_{SR} P_{SE} P_G \quad (13)$$

where P_C(Vent. release) is the probability that contamination will be released through the venting system.

$P_R = P_{ins}$

where P_{ins} is the probability of instability occurring

$P_{SR} = 10^{-4}$

$P_{SE} = 1$

$P_G = 10^{-7}$

and N' = N x P (#/unit volume) x Volume released

where N is the number of interior surface organisms, N = 4.6×10^4 from Table 3.

P (#/unit volume) is the probable fraction of the total number of viable particulates released per unit volume of gas relased.

P_C(Vent. release) = 4.6×10^{-7} $XP_{ins}XP$ (#/unit volume) x Volume released.

If the value of everything to the right of 4.6×10^{-7} were 1, this would be a marginal situation. This is highly unlikely and a value of 10^{-1} - 10^{-2} does not seem unreasonable. Therefore, this contamination mode should not present serious problems.

2) Events which occur after the peak heating.

The main difference between release of organisms prior to or during peak heating and after the peak heat was considered in para. A21.2.1 under Non-Nominal Events. Briefly, organisms released in the former case experience higher heat rates and therefore a P_{SR} of 10^{-4} has been used. Organisms released after peak heating, if in a viable condition prior to release will remain that way. Therefore, P_{SR} is replaced by P_{SA}, the probability that the organism survives entry heating while in place.

a) Extension of parachutes, deployable fins, other aerodynamic devices.

The equation describing contamination by this mode is:

$$P_C(D(Enc)) = NP_R P_{SA} P_{SE} P_G \quad (14)$$

where all symbols have been defined previously.

The values for encapsulated burden are given in Table 1 in the column for late release, and N is given in para A2.1.2.2(a) as 3.6×10^6 organisms.

Therefore, $P_C(D(Enc)) = 3.6 \times 10^{-5}$

This is a marginal result. However, several assumptions have been made. First, that all N organisms are released late; this is overly conservative. Secondly, that all of the burden can be treated as encapsulated burden as far as the response to entry heating. Some of the burden which is considered to be encapsulated late in the entry may actually not be at this point. Therefore, the actual value of $P_C(D(Enc))$ may be reduced below the 3.6×10^{-5}, possibly by an order of magnitude. The result would still be marginal indicating that some microbial burden reduction technique would have to be used on these components to achieve about a one to two log reduction in burden count.

b) Cracking of the Heat Shield. The situation here is the same as prior to the peak heat pulse except that any viable organisms released would not be killed by entry heating. It was established in para. A2.1.2.2.(b) that heat shield could be considered to be sterile due to the temperature it received during the peak entry heating. The equation which could apply to this situation is:

$$P_C(H.S.\ (Enc)) = NP_R P_{SA} P_{SE} P_G \qquad (15)$$

where in this case P_R is considered to be 1.0 because the unique situation of cracking is being considered. In other words, the probability that cracking occurs is assumed to be one.

P_{SA} is a small number because of the high temperature achieved by the heat shield from entry heating.

$P_{SE} = 1$ and $P_G = 10^{-7}$.

Therefore $P_C(H.S.\ (Enc)) = 10^{-7} \times P_{SA} \times N$

if $N \times P_{SA}$ is 1.0 this mode of contamination will not be a problem. Since a heat shield may be heated during manufacture burden levels can be kept low. If N is kept to 10^3 to 10^4 and P_{SA} is in the range of 10^{-3} to 10^{-4} then this mode will not be a problem. The value of P_{SA} is certainly within reason, given the high temperature to which the heat shield will be subjected.

Equation (15) can also be applied to the powder in the honeycomb structure where $P_R = 1$, $P_{SR} = 1$,

$P_{SE} = 1$, $P_G = 10^{-7}$ and $N = 10^5$, where N was derived in para. A2.1.2.2(b). P_{SA} is undetermined here because the powder remains insulated from the heat pulse. Therefore, the probability of contamination due to release of the powder is $10^{-2}P_{SA}$, where P_{SA} s in the range 1.0 to 0.1.

Therefore, the quarantine constraint will be exceeded unless N is reduced to 10 or less. Therefore, the powder must be subjected to microbial reduction techni niques. This assumes, of course, that the probability of this mode of release is 1.0. If heat shield cracking has the lower probability than 1.0, the allowable value of N will increase proportionately.

c) Release of Contamination Through the Probe Venting System. Equation (15) can be modified to apply to this case by replacing P_{SR} by P_{SA}, therefore:

$$P_C(\text{Vent release (late)}) = N'P_RP_{SA}P_{SE}P_G \qquad (16)$$

where the quantities were previously defined in para. A2.1.2.2(c) and equation (16) becomes

$$P_C(\text{Vent release (late)}) = P_{ins}NP(\#/\text{Unit vol}) \times \text{Vol. released} \times 1 \times 10^{-7} \qquad (17)$$

where as defined in Section 2-a-(3)

$P_R = P_{ins}$ = the probability of an instability

$P_{SA} = 1$ since the interior of the probe does not experience any significant additional heat, $P_R = 1$ and $P_{SE} = 1$ and N' has been replaced by N x P (#/Unit vol) x Vol released,

where P(#/Unit vol) is the probable fraction of the total number of viable particulates released per unit of volume released. Vol released is the actual volume of gas released.

Therefore, in order that this not be a significant contamination mode the quantity multiplying 1×10^{-7} should be 1 or less. It is estimated and given in Table 3 that the interior surface burden would be 4.6×10^4. Therefore, without any heating of the probe interior N would be 5×10^4. Unless P_{ins} is less than 10^{-5} or 10^{-6} burden reduction will be necessary.

Several potential solutions are possible. One would be to introduce a filter into the venting system which would prohibit organisms from passing through. One type of filter currently in use, the HEPA (High Efficiency Particulate Air) Filter filters out particulates

.3μ or larger with a 99.97% efficiency. It is probable that should filtration be chosen as the solution, a filter could be found. It would also have to be capable of filtering out particulates .3μ or larger. Other potential solutions include one way valves or "flappers". In any case the system used to stop organisms from passing out through the vent system must also allow the appropriate pressure differential to be maintained across the surface of the probe. The burden reduction efficiency of the system would be a function of the values of P_{ins}, P(#/Unit vol) Vol released, but in no case would have to be greater than 10^{-5} - 10^{-6}, and most probably would have to be 10^{-3} or less. By way of comparison use of one HEPA filter (if it applies in this case) would provide a reduction of 3×10^{-4}.

d) Release of Viable Particulates Through Instrument Sensor Ports. Instrument sensor deployment occurs after the probe reaches an atmospheric density of 2×10^{-5} g/cm^3. At this point any viable particulates released will not be subjected to any appreciable entry heating.

$$P_C(SD(IS)) = NP_R P_{SA} P_{SE} P_G \qquad (18)$$

Consider some of the details of the sensor deployment. Some of the sensors are deployed through the heat shield and into the local atmospheric environment at the same time a plug is ejected. This is a requirement of four instruments. A sampling tube will be projected through the nose center line. Gas samples are routed to the mass spectrometer and the gas chromatograph. The sampling tube also serves as a total pressure plenum for pressure profile measurements. The mass spectrometer-gas chromotograph sampling tube which is capped, pushes through the heat shield where the cap separates.

There is a temperature sensing unit which must penetrate the heat shield in order to let the high velocity flow over dual platinum wires.

The nephelometer and net flux radiometer require jettisonable panels in the heat shield, however, only the net flux radiometer extends outside the mold line. The nephelometer views outward and radially through a transparent cover.

The energetic particle detector, located in the forward portion of the probe, views aft through the heat shield.

A number of contamination modes can be envisaged as arising from the deployment of the sensor instrumentation. One mode is the carrying of organisms to the exterior of the probe by the projection of instrumentation, tubes, and so forth. Since these components are inside the heat shield during the heat pulse, they will not be heated significantly. Another mode of organism release may occur by gas exchange during the introduction of a gas sample into the instrument through a sampling tube. Any plugs which are released may be a contamination source. They could be sterilized prior to launch.

The contamination equation covering any of these modes is:

$$P_C(SD(Burden)_i) = NP_R P_{SA} P_{SE} P_G \qquad (18)$$

where the symbols have been previously defined and the burden type designated by i can represent any of the three types; mated, interior burden, and encapsulated burden.

The value of P_R depends on the burden type and release mode. Interior surface burden on an instrument or tube has a high probability of being released if projected to the outside of the heat shield. Mated or encapsulated burden has a much lower probability. Therefore, in the former case $P_R = 1.0$, while in the latter it would probably be negligibly small unless a piece or component such as the cap was released in which case organisms could be released later. P_R in the case of gas interchange is really composed of the probability of releasing an organism into the gas stream and then of having a gas interchange. The combined probability is probably small, probably less than or equal to 10^{-2}. Therefore, in summary assume that:

$P_R = 1.0$ for surface burden located on the outside of the tube or instrumentation exposed to the atmosphere.

P_R = neg. for mated or encapsulated burden.

$P_R = 10^2$ for release of organisms due to gas interchange.

$P_{SA} = 1$ for all cases

$P_{SE} = 1.0$ for all cases

$P_G = 10^{-7}$

Therefore, P_C for the three situations associated with P_R above in the same order is:

$P_C = N \times 10^{-7}$

$P_C = N \times \text{neg.}$

$P_C = N \times 10^{-9}$

Therefore, the exterior burden located on tubes, instrumentation and so forth should be less than 100 organisms, in order to satisfy the probe P.Q. constraint. This will mean some kind of cleanliness control or decontamination.

The mated or encapsulated burden is not a significant factor in these contamination modes.

The burden which would be released during the gas interchange, which would be interior surface burden would have to be 10^2 or less. Considering the mode or release and small amount of gas involved standard cleanliness practices will ensure that mode will satisfy the PQ constraints.

e) Probability of Contamination due to Probe Break-up Because of Atmospheric Pressure. The probe will be designed to survive to some pressure in the atmosphere. The nominal mission will collect data to some point. The probe may survive for some distance after that into the atmosphere or it may not.

The following aspects of probe break-up are critical. If the point of break-up occurs in the biozone or just after passage particulates may be released into the biozone. If the probe is released far down in the atmosphere particulates may not reach the biozone containing viable organisms. In both cases the probability of survival and growth depends on atmospheric circulation patterns. The mode of break-up will be due to the inability of the interior pressure to equilibrate resulting in structural failure of the probe body. The microbial release mechanisms and the analysis of the probability of contamination when break-up occurs near the biozone are similar to those for contamination due to break-up of the probe because a venting system failure (i.e., non-nominal failure) occurs after peak heating. This is discussed in para. A2.1.2.1. The analyses of break-up deep into the atmosphere will need the further addition of the probability of atmospheric circulation transporting an organism up into the biozone. This is beyond the scope of this report. However studies are currently underway in the Natural Space Environmental Task to determine transport possibilities.

A3. CONCLUSIONS

Applying standard planetary quarantine analysis techniques used for Mariner, Pioneer, and Viking Programs to a proposed Outer Planets Orbiter/Probe (Jupiter) Mission leads to the following conclusion(s) for the Probe.

1) Standard spacecraft cleanliness and contamination control techniques will suffice to satisfy quarantine constraints for the majority of the Probe equipment.

2) Some selected spacecraft components and materials such as insulation materials and ejectable plastic caps may require some degree of dry heat sterilization.

A4. REFERENCES

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2. Prelaunch Analysis of the Probability of Planetary Contamination, Vol. 1, PR-3701060, Martin Marietta Corp., Denver Division, December 5, 1974.

3. Hall, L. B., "Revised and New Planetary Quarantine Policies", NASA SL Letter, August 24, 1971.

4. Mariner Jupiter/Saturn 1977 Planetary Quarantine Plan, PD618-114, August 23, 1973.

5. Unpublished report summarizing outer planet atmospheric entry heating studies.

APPENDIX B

EFFECT OF HIGH ENERGY ELECTRONS ON MICROORGANISMS

B1. INTRODUCTION

This work describes the effect of high energy electrons on survival of microorganisms associated with unsterile spacecraft. it is a subtask of a larger task concerned with determining the consequences of solar, cosmic and planetary trapped radiations on spacecraft decontamination requirements. With fly-by missions now planned for Jupiter and Saturn and possible Jupiter orbiters and probes, the planetary trapped radiation belts represent environments lethal to microorganisms that could reduce pre-launch spacecraft decontamination requirements. The major components of these radiation belts are electrons and protons. This work evaluates possible biological effects of the electron component of these belts by subjecting several spacecraft microbial isolates to different energies, exposures, and dose rates and then investigating models that describe the microbes' survival as a function of the radiation insult. Engineering models for estimating microbe survival in high energy electron environments are presented.

The microbe property investigated here is reproductive integrity. The ability to reproduce is the most radiosensitive function of the cell and is the property usually investigated in radiobiology. In this present work it is the property of primary concern, since if a cell is incapable of division, contamination is inconsequential. Reproductive death is measured quantitatively as the ability of a microbe to form a clone under optimum conditions. For application to planetary quarantine, it is assumed that a cell incapable of division under optimum conditions would be incapable of division under conditions of planetary contamination.

Radiobiologically, the radiation damage that causes reproductive death is typically ascribed to inactivation of a sensitive target, most probably DNA. The physical interactions of radiation with biological materials are complex, and the mechanisms linking initial physical interactions to final biological damage are largely unknown. Therefore survival models were investigated with two objectives: first, to describe the survival as well as possible; and second, to see whether models with physical significance could be developed.

B2. EXPERIMENTAL MATERIALS AND METHODS

For investigating radiation effects on possible spacecraft contaminants, over 200 different organism types were recovered and isolated from different stages of Mariner Mars 71 spacecraft assembly. Of these, twelve were used in the investigations. Of the twelve microbes, 9 were sporeforming and 3 vegetative. Two routinely cultured organisms, Bacillus subtilis var. niger and Staphyloccocus epidermidis, were also tested as comparison organisms.

The test apparatus is described in reference 1 and the microbiological procedures in reference 2. Test microbes were exposed to high energy electron beams as follows:

1) A test sample comprised of four identical subsamples is prepared. Each subsample consists of 14 planchets each innoculated with one of the test microorganisms (approximately 10^5 organisms for sporeformers or 10^6 for non-sporeformers).

2) The sample is mounted in the test apparatus. Two subsamples are mounted on an exposure plate and two on a non-exposed control plate. One exposed and one non-exposed subsample are maintained at +20°C. The other exposed and non-exposed subsamples are maintained at -20°C. The entire sample is maintained at 10^{-6} torr.

3) The organisms are exposed: the independent variables are temperature, energy, flux, dose. Table B1 is the test matrix for the data analyzed in this report.

4) Each cell of the test matrix is repeated 4 times.

5) After exposure the microbes are assayed for viability, i.e. their ability to form clones. Survival Fraction = (Number of viable microbes in exposed sample)/(Number of viable microbes in unexposed sample).

Table B1. Test Matrix for Microbe Radiosensitivity

Energy, MeV		Flux, $cm^{-2}sec^{-1}$	Dose Rate, (tissue) rad sec^{-1}	Fluence, cm^{-2}	Dose, krad (tissue)	
Nominal	Actual				Nominal	Actual
0.7	0.6	9.1 (8)	30	4.5 (12)	150	145
		9.1 (9)	300	9.1 (12)	300	290
		9.1 (10)	3000	1.4 (13)	450	440
2	1.93	1.0 (9)	30	4.85 (12)	150	145
		1.0 (10)	300	9.70 (12)	300	290
		1.0 (11)	3000	1.45 (13)	450	440
12	10.65	8.4 (8)	30	4.10 (12)	150	145
		8.4 (9)	300	8.20 (12)	300	290
		6.5 (10)	2200	1.24 (13)	450	440
25	23.40	7.0 (8)	30	3.50 (12)	150	145
		7.1 (9)	300	7.00 (12)	300	290
		5.4 (10)	2200	1.07 (13)	450	440

B3. DATA ANALYSIS

The test data were prepared for analysis by averaging the results of the 4 observations at each data point and computing an estimate of the standard deviation of the mean survival fraction. The mean survival fraction weighted by the inverse of its standard deviation was the data used to develop and evaluate models of radiation sensitivity.

Each model's ability to fit the data was evaluated quantitatively. The criterion for judging the goodness of fit for a particular model is the weighted root-mean-square error.

$$\text{RMS Error} = \sqrt{\frac{1}{\upsilon} \sum_{i=1}^{N} \frac{1}{\sigma_i^2} \left(Y_i - f(x_i, p_i)\right)^2} \qquad (1)$$

υ is the number of degrees of freedom in the data, Y_i is the mean value of the ith data point, $f(x_i,p_i)$ is the value of the model at that point and σ_i is the standard deviation associated with the ith point. Two computer programs, one a linear regression program in the JPL library and the other a non-linear regression fitting program developed as part of this task, are used to evaluate models. Both programs determine model parameters that produce the minimum weighted RMS error for the model under investigation.

The capabilities of several models to fit the data have been investigated. The origin and capabilities of each model are discussed in the next section. Reference 2 presents an analysis of variance of the data that indicates all the independent variables, organism, temperature, energy, flux and fluence, have significant effects on the survival fraction. Therefore, an attempt was made to employ these variables explicitly in some of the models. However, the analysis of variance is not robust with respect to the assumption of equal sample variance for each data point (i.e. it is not capable of determining whether the observation is caused by a functional dependence or by varying uncertainties introduced as the result of changing the variable) and in subsequent model evaluation no simple functional dependence on energy or flux was apparent within the range of the experiment.

Three significant conclusions may be based on behavior of the data. First, the non-sporeforming microbes are significantly more radio sensitive than the sporulated microbes. Second, each model was investigated twice, once with survival fraction as a function of dose and once as a function of fluence.[1] For every model the RMS error was smaller with fluence as the independent variable. This behavior indicates that radio sensitivity is proportional to electron fluence rather than an average energy density. Third, as microbe temperature increases, the survival fraction at a given fluence decreases. This behavior indicates that the temperature effect is not the result of a heat activated repair mechanism, but possibly an accelerated cell cycle.

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[1]Green-Burki model excepted.

B4. RESULTS[1]

B4.1 Linear Model

As microbes are irradiated it is reasonable to assume that the number of microbes "killed" is proportional to the total number of viable microbes present and the electron fluence, i.e. $dN = -\alpha N d\Phi$ where N is the number of viable organisms and Φ is the fluence. The solution to this equation is

$$N = N_o e^{-\sigma'\Phi} \qquad \text{or in linear form} \qquad (2)$$

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi. \qquad (3)$$

In the absence of other factors (i.e. at constant dose rate, temperature, flux, etc.) this exponential behavior is observed in Figure B1. The figure is a graph of the survival data for Bacillus subtilis var. niger (circles and triangles) and the model (solid line). Parameter values and RMS error for the linear model for each organism are given in Table B2.

Table B2. Parameters and RMS Error for Linear Model

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi + K$$

Organism	σ ($\times 10^{-13}$ cm^2)	K ($\times 10^{-5}$)	RMS Error ($\times 10^{-2}$)
1	1.87	1.65	6.76
2	1.87	4.98	6.95
8	1.82	2.83	7.14
9	1.80	2.91	6.92
11	1.40	1.83	4.95
12	1.47	2.73	7.17
13	1.47	3.72	5.47
16	1.40	3.80	5.83
18	1.36	1.77	5.23
BSN	2.09	4.55	8.04
4	3.51	-21.7	32.4
5	2.22	6.67	13.4
19	3.58	-11.3	25.7
SE	3.84	-74.9	32.3
Spore Mean	1.06	7.66	5.65
Vegetative Mean	3.00	-3.49	1.63

Figure B1 also illustrates a modified linear model shown as dashed lines. The model, equation 4, incorporates a temperature term.

$$\text{Log}_{10}(N/N_o) = -\sigma\,(1 + K_1\Delta T)\;\Phi + K_2 \qquad (4)$$

This model provided a significant improvement in fit to the data over the simple linear model. Parameter values and RMS error are given in Table B3.

Table B3. Parameters and RMS Error for Modified Linear Model

$$\text{Log}_{10}(N/N_o) = -\sigma\,\left(1 + K_1(T-273)\right)\;\Phi + K_2$$

Organism	σ ($\times 10^{-13}$ cm^2)	K_1 ($\times 10^{-3}$ °K^{-1})	K_2 ($\times 10^{-4}$)	RMS Error ($\times 10^{-2}$)
1	1.87	2.50	0.997	6.59
2	1.86	4.56	0.997	6.15
8	1.81	2.52	0.997	7.00
9	1.77	5.64	0.996	5.83
11	1.40	2.11	0.992	4.90
12	1.46	3.77	0.994	6.99
13	1.46	3.31	0.994	5.25
16	1.39	3.08	0.994	5.70
18	1.36	2.55	0.991	5.16
BSN	2.06	5.03	0.998	6.84
4	3.48	1.62	0.998	32.5
5	2.12	8.99	0.999	10.9
19	3.56	1.14	0.999	25.8
SE	3.82	1.15	0.998	32.4
Spore Mean	1.06	4.99	1.00	7.50
Vegetative Mean	2.91	5.02	1.00	14.4

B4.2 Quadratic Model

The dual radiation action theory of Rossi and Kellerer (reference 3) gives a quadratic form for production of elementary lesions ϵ in biologic materials.

$$\epsilon = \alpha\,(K\Phi + \Phi^2) \qquad (5)$$

If the number of microbes "killed" is proportional to the total number of viable microbes and the rate of lesion production, then $dN = Nd$. The solution to this equation is

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi + \gamma\Phi^2 + K \qquad (6)$$

This model gave improved results over the linear model for several of the organisms studied. Table B4 lists parameter values and RMS error.

Table B4. Parameters and RMS Error for Quadratic Model

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi + \gamma\Phi^2 + K$$

Organism	σ ($\times 10^{-13}$ cm^2)	γ ($\times 10^{-27}$ cm^4)	K ($\times 10^{-5}$)	RMS Error ($\times 10^{-2}$)
1	1.61	-2.11	-1.04	6.70
2	1.26	-4.95	-1.23	6.46
8	1.48	-2.72	-0.539	7.03
9	1.53	-2.14	0.312	6.86
11	1.12	-2.34	-0.710	4.85
12	1.12	-2.87	-0.431	7.07
13	1.01	-3.81	-0.452	5.16
16	0.920	-3.93	-0.428	5.54
18	1.11	-2.11	-0.493	5.16
BSN	1.60	-3.93	-0.815	7.77
4	5.28	14.0	11.8	30.6
5	1.54	-5.45	-0.586	13.1
19	4.73	8.91	10.2	24.8
SE	6.74	22.8	9.03	22.4
Spore Mean	0.036	-8.50	0.627	4.36
Vegetative Mean	3.09	0.655	-2.31	16.4

An ad hoc term for temperature dependence was used to modify the quadratic model in equation 7.

$$\text{Log}_{10}(N/N_o) = -\sigma(1 + K_1\Delta T)\Phi + \gamma(1 + K_2\Delta T)\Phi^2 \qquad (7)$$

This model gave the best fit to the data for all organisms. It is illustrated for Bacillus subtilis var. niger in Figure B2 and parameter values and RMS error are tabulated in Table B5.

Table B5. Parameters and RMS Error for Modified Quadratic Model

$$\text{Log}_{10}(N/N_o) = - (1 + K_1(T-273)) - (1 + K_2(T-273))^2$$

Organism	σ (X 10^{-13} cm^2)	γ (X 10^{-27} cm^4)	K_1 (X 10^{-3} $^oK^{-1}$)	K_2 (X 10^{-3} $^oK^{-1}$)	RMS Error (X 10^{-2})
1	1.44	3.37	5.40	-4.81	6.57
2	1.23	5.06	5.42	2.27	5.62
8	1.28	4.32	5.08	-3.68	6.95
9	1.33	3.52	5.43	5.46	5.85
11	1.09	2.58	4.98	-7.13	4.80
12	1.06	3.31	5.21	-0.888	6.89
13	1.03	3.60	5.00	-0.461	4.94
16	0.987	3.37	5.10	-1.85	5.41
18	1.06	2.43	4.99	-5.63	5.09
BSN	1.36	5.57	5.34	3.78	6.66
4	3.06	3.18	3.55	-7.51	33.7
5	1.63	3.99	11.5	-0.144	10.7
19	2.79	5.62	3.29	-6.33	27.7
SE	3.42	2.30	2.36	-5.39	34.7
Spore Mean	0.326	6.19	8.78	-0.506	4.33
Vegetative Mean	2.61	2.10	6.15	-2.79	14.7

B4.3 Green-Burki Model

The model presented in reference 4 was adapted from population kinetics to incorporate a simple repair or recovery term into the linear model. The differential equation that defines the model is

$$dN = -(\alpha N - \beta N^2)d\Phi \qquad (8)$$

The change in number of organisms or the number damaged is proportional to the number of viable organisms present and the dose. From the number damaged the fraction of those cells that recover is subtracted. This number of recovered cells is assumed to be proportional to the square of the number of organisms present. The solution to this equation is

$$N = K_1/(e^{K_o\Phi+K_1}-1) \qquad (9)$$

This model fits curves with "shoulders" but the data did not exhibit this behavior to any extent and other models gave better results. The model is shown in Figure B3, and parameter values and RMS errors are tabulated in Table B6.

Table B6. Parameters and RMS Error for Green-Burki Model

$$N = K_1/(e^{K_o\Phi}+K_1-1)$$

Organism	K_o ($\times 10^{-13}$ cm^2)	K_1	RMS Error ($\times 10^{-2}$)
1	5.90	0.719	20.4
2	5.53	0.636	17.9
8	6.02	0.961	18.6
9	5.96	0.844	19.7
11	4.58	0.821	16.2
12	5.15	0.673	20.4
13	4.91	1.01	17.2
16	4.79	1.01	17.8
18	3.52	-0.088	15.8
BSN	5.71	0.255	17.9
4	14.1	0.800	28.0
5	6.72	-0.072	24.2
19	11.6	0.796	26.1
SE	20.2	0.800	30.7
Spore Mean	3.86	0.827	7.97
Vegetative Mean	10.9	0.792	16.1

B4.4 Repair Model:

This model was derived heuristically and includes a temperature, flux and fluence dependent repair term.

$$dN/d\Phi = -\sigma N + \frac{Ke^{-\gamma\Phi}}{T \ln\phi} N\Phi \qquad (10)$$

The rate of "damage" is proportional to the number of organisms present and the dose, this accounts for the first term. The second term is the rate of repair of the damaged cells.

The form for the repair is based on the following assumptions. The repair rate is proportional to the number of damaged, reparable cells. The number of damaged cells is proportional to the rate of damage times the fluence, $dN/d\Phi \times \Phi$. If repair is small $dN/d\Phi$ is approximately proportional to -N. The number of reparable cells (e.g. single strand DNA breaks) decreases with increasing fluence as $e^{-\gamma\Phi}$ so that the number of reparable cells is proportional to $N\Phi e^{-\gamma\Phi}$. The repair rate is also proportional to the inverse of the logarithm of flux; as the flux increases the rate of repair slowly decreases because the increased flux results in increased irreparable damage (e.g. double strand DNA breaks). Finally the repair rate term is divided by temperature to take into account the observed temperature dependence.

The solution to this equation is

$$\mathrm{Log}_{10}(N/N_o) = -\sigma\Phi + \frac{K}{T \ln\Phi}\left[1 + e^{-\gamma\Phi}(-\gamma\Phi - 1)\right]. \qquad (11)$$

This model is not capable of fitting the data as well as the quadratic model. It is illustrated in Figure B4 and parameters and RMS error are given in Table B7.

B4.5 Other Models

There are many possible survival models that were not investigated; they fall generally into two classes. The first class consists of functions and higher order curves capable of improved quantitative fit to the data but that have no physical interpretation. These models were excluded because they add complexity but are insufficiently general to increase the information content of the model. The second class includes the models appearing in the literature that attempt to describe the relative biological effectiveness of radiation as a function of LET (e.g. those of Katz, Payne and Garrett). For electrons in a narrow range of energies such as investigated in this report these models reduce to models similar to those presented above.

Table B7. Parameters and RMS Error for Repair Model

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi + \frac{K}{T\ \ln}\left[1 + e^{-\gamma\Phi}(-\gamma\Phi - 1)\right].$$

Organism	σ ($\times 10^{-13}$ cm^2)	γ ($\times 10^{-14}$ cm^2)	K	RMS Error ($\times 10^{-2}$)
1	1.96	9.24	6.75	6.82
2	1.96	9.34	6.75	7.01
8	1.90	9.29	6.82	7.20
9	1.88	9.31	6.89	6.96
11	1.49	9.68	7.03	5.00
12	1.56	9.65	7.03	7.24
13	1.55	9.56	6.95	5.54
16	1.48	9.76	6.95	5.91
18	1.45	9.65	7.03	5.29
BSN	2.17	9.22	6.75	8.08
4	3.73	11.3	15.96	32.5
5	2.30	9.58	6.89	13.4
19	3.76	11.9	11.59	25.8
SE	4.29	8.24	49.40	30.9
Spore Mean	1.23	*	3.56	5.12
Vegetative Mean	3.09	9.08	6.69	16.3

*1.56×10^{17}

B5. CONCLUSIONS

B5.1 Limitations

The models are all higly suspect outside the range of the data for the following reasons. First the temperature and flux ranges investigated are very limited. Second, while the energy effects are small the results of a parallel subtask (Effect of Solar Wind on Microorganisms) indicate they become very large as the energy decreases, particularly below about 15 keV. Finally, as the models are phenomenological and not physical, there is no assurance that they are valid outside the fluence, flux, temperature and energy ranges investigated and extrapolation could yield misleading estimates.

B5.2 Planetary Quarantine Estimation

Considering the models evaluated the modified quadratic model, equation 7, had the lowest RMS error for all organisms[2] and therefore is the model recommended for planetary quarantine microbial burden estimates.

High, nominal and low survival engineering models for estimating microbe survival in high energy electron environments were constructed. These models are illustrated for the comparative organism Bacillus subtilis var. niger in Figure B5. The nominal model is the modified quadratic model of Section B4.2. The high and low survival models were constructed by fitting a parabola to the extreme (highest and lowest) survival observed for each fluence. The area between these two models therefore includes most of the data observed and observations outside the area have a low probability of occurrence. The high nominal and low survival model parameters are given in Tables B8, B5, and B9 respectively.

Table B8. Parameters for High Survival Model

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi - \gamma\Phi^2 + K$$

Organism	σ ($\times 10^{-13}$ cm^2)	γ ($\times 10^{-27}$ cm^4)	K ($\times 10^{-3}$)
1	1.09	4.14	-5.94
2	0.643	7.11	-6.43
8	1.31	1.28	-0.193
9	0.988	3.10	-1.88
11	0.751	3.05	-1.06
12	0.902	1.89	3.29
13	0.779	3.16	1.90
16	0.370	6.35	-0.439
18	0.403	5.34	-3.01
BSN	1.26	3.13	1.56
4	1.88	0.360	11.2
5	0.659	5.25	-1.49
19	2.00	4.31	7.67
SE	2.16	5.64	-4.40
Spore Mean	0.142	8.05	2.86
Vegetative Mean	2.24	-1.82	-7.70

[2]Organism 4 excepted. Survival data for this organism included several observations below the detection limit that made model comparison difficult.

Table B9. Parameters for Low Survival Model

$$\text{Log}_{10}(N/N_o) = -\sigma\Phi - \gamma\Phi^2 + K$$

Organism	σ ($\times 10^{-13}$ cm^2)	γ ($\times 10^{-27}$ cm^4)	K ($\times 10^{-3}$)
1	2.05	1.02	-7.76
2	1.82	3.00	-3.90
8	1.55	4.84	-10.2
9	2.01	1.36	-1.23
11	1.45	2.05	-4.24
12	1.51	2.41	-7.68
13	1.11	5.29	-9.06
16	1.24	3.73	-3.74
18	1.79	-1.16	-4.56
BSN	2.19	1.94	0.028
4	7.68	-28.9	47.0*
5	2.41	3.38	-10.1
19	6.37	-19.5	40.9*
SE	8.47	-34.7	9.23*
Spore Mean	0.266	8.83	1.21
Vegetative Mean	4.34	-6.21	1.41*

*Data for these organisms included several observations below the detection limit making it difficult to optimize model parameters. Data points below the limit were assigned a 10^{-5} survival fraction.

B6. REFERENCES

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4. Cohen, L.; "A Comment on the Note by Green and Burki on Survival Curves," Radiation Research, Vol. 64, p. 393, 1975.

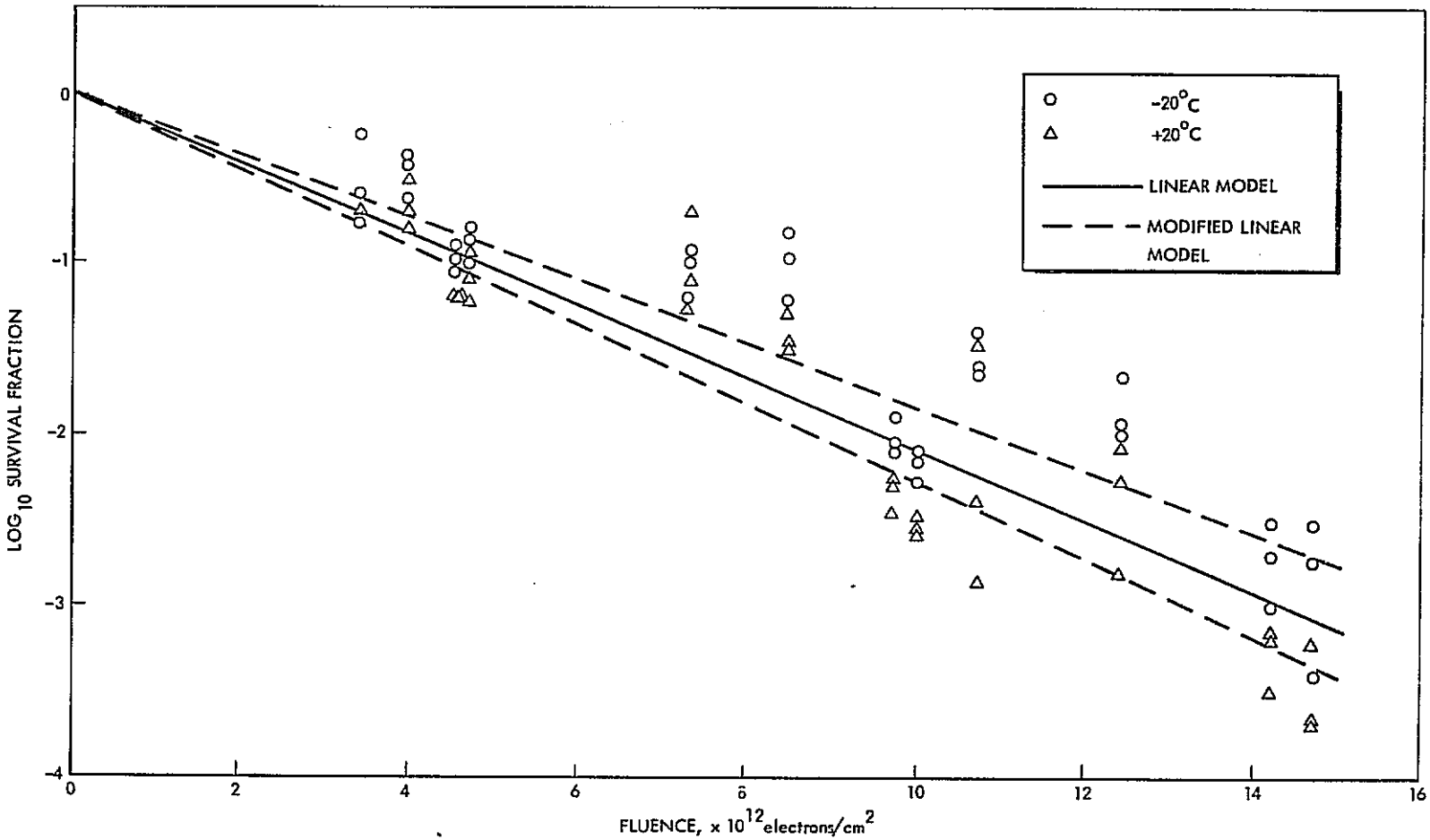

Figure B-1 Linear Model

For Bacillus Subtilis Var. Niger

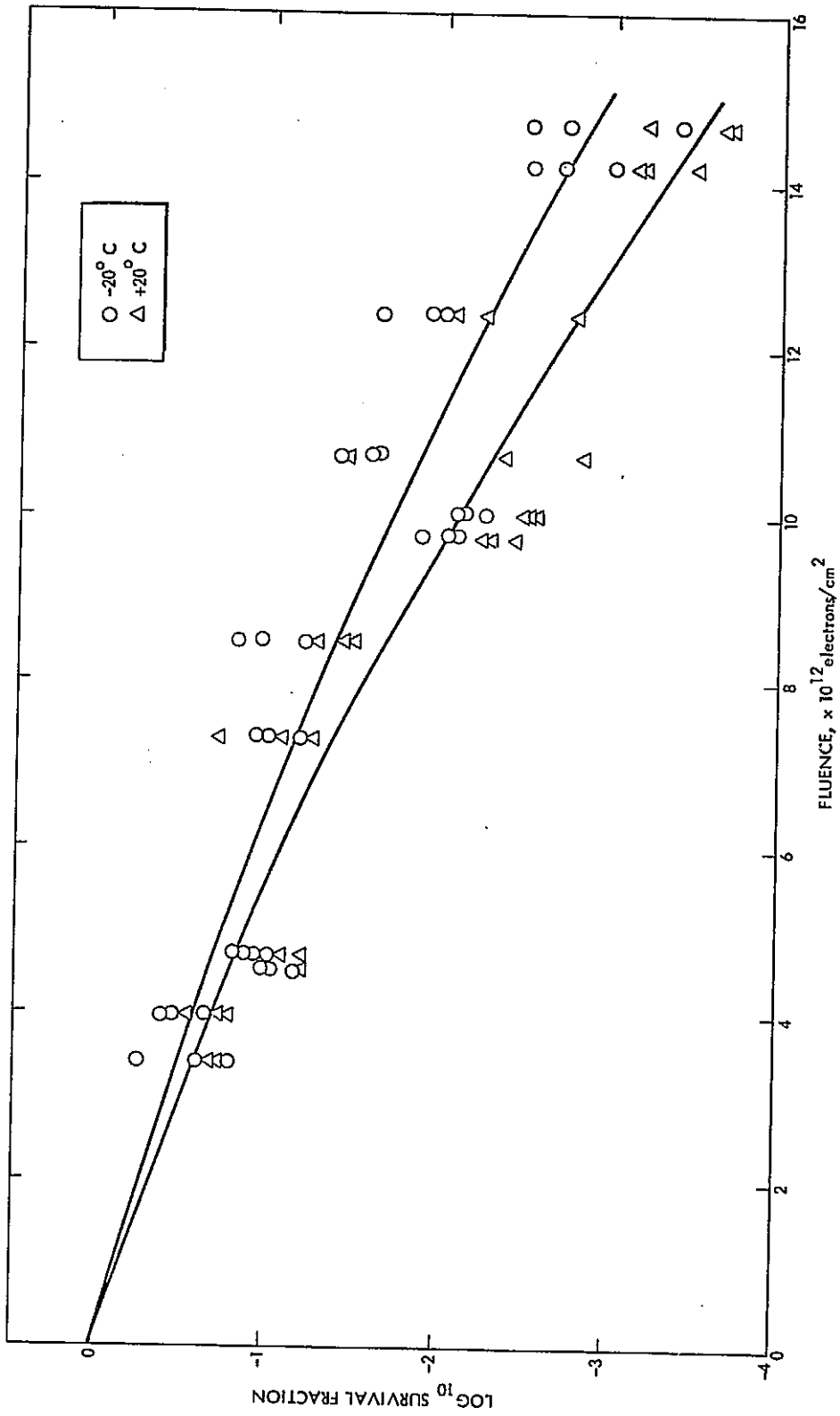

Figure B-2 Modified Quadratic Model
For Bacillus Subtilis Var. Niger

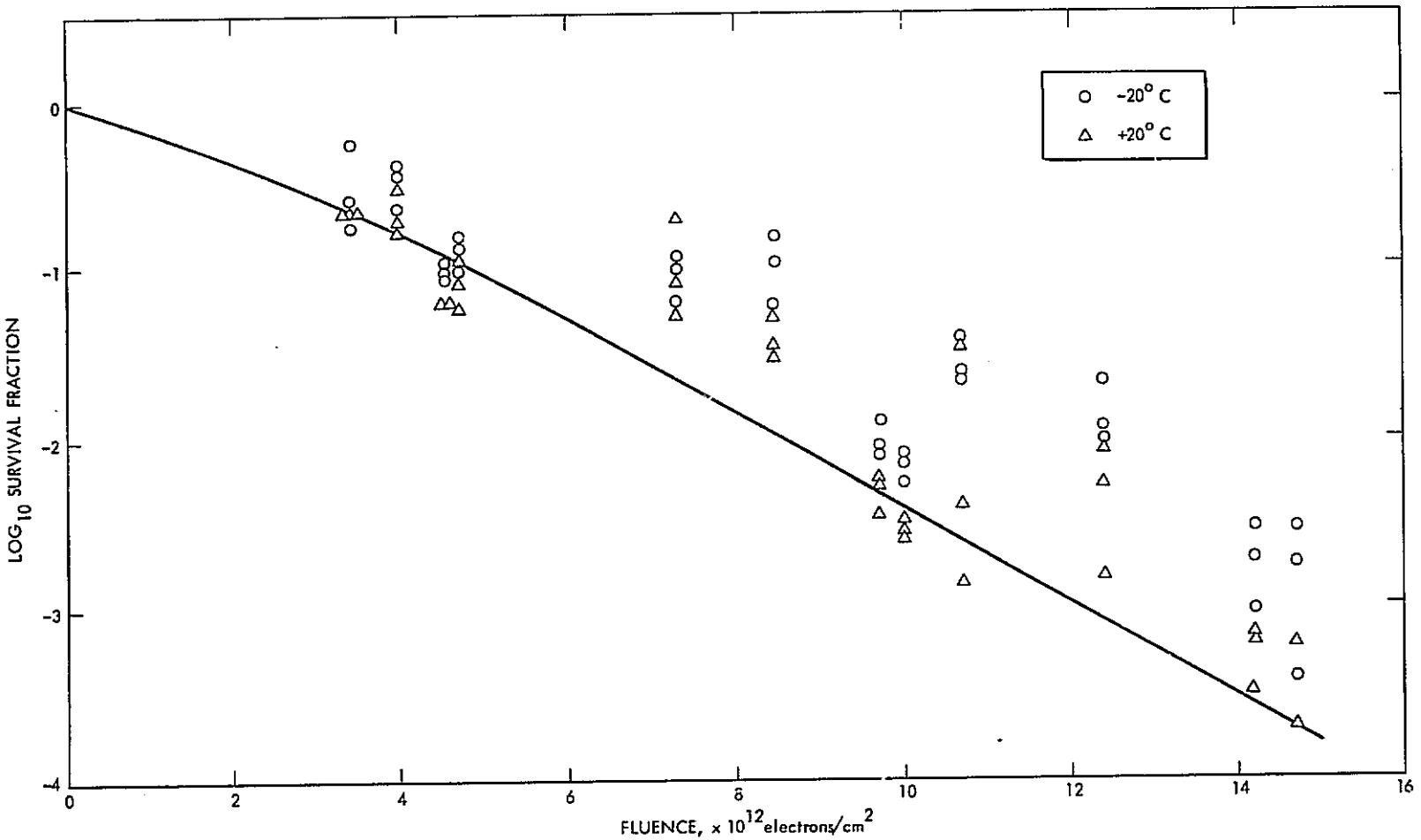

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Figure B-3 Green-Burki Model
For Bacillus Subtilis Var. Niger

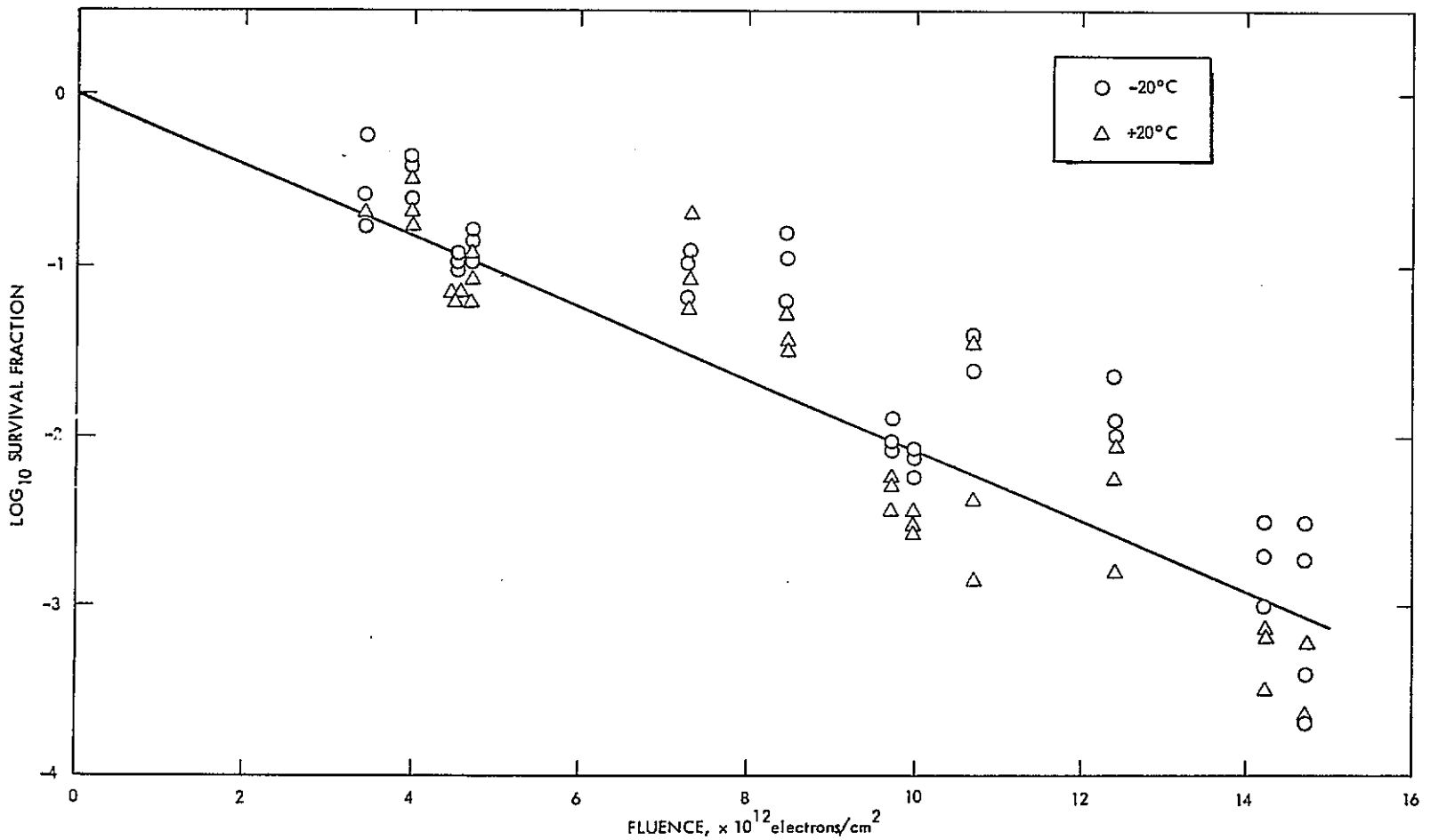

Figure B-4 Repair Model
For Bacillus Subtilis Var. Niger

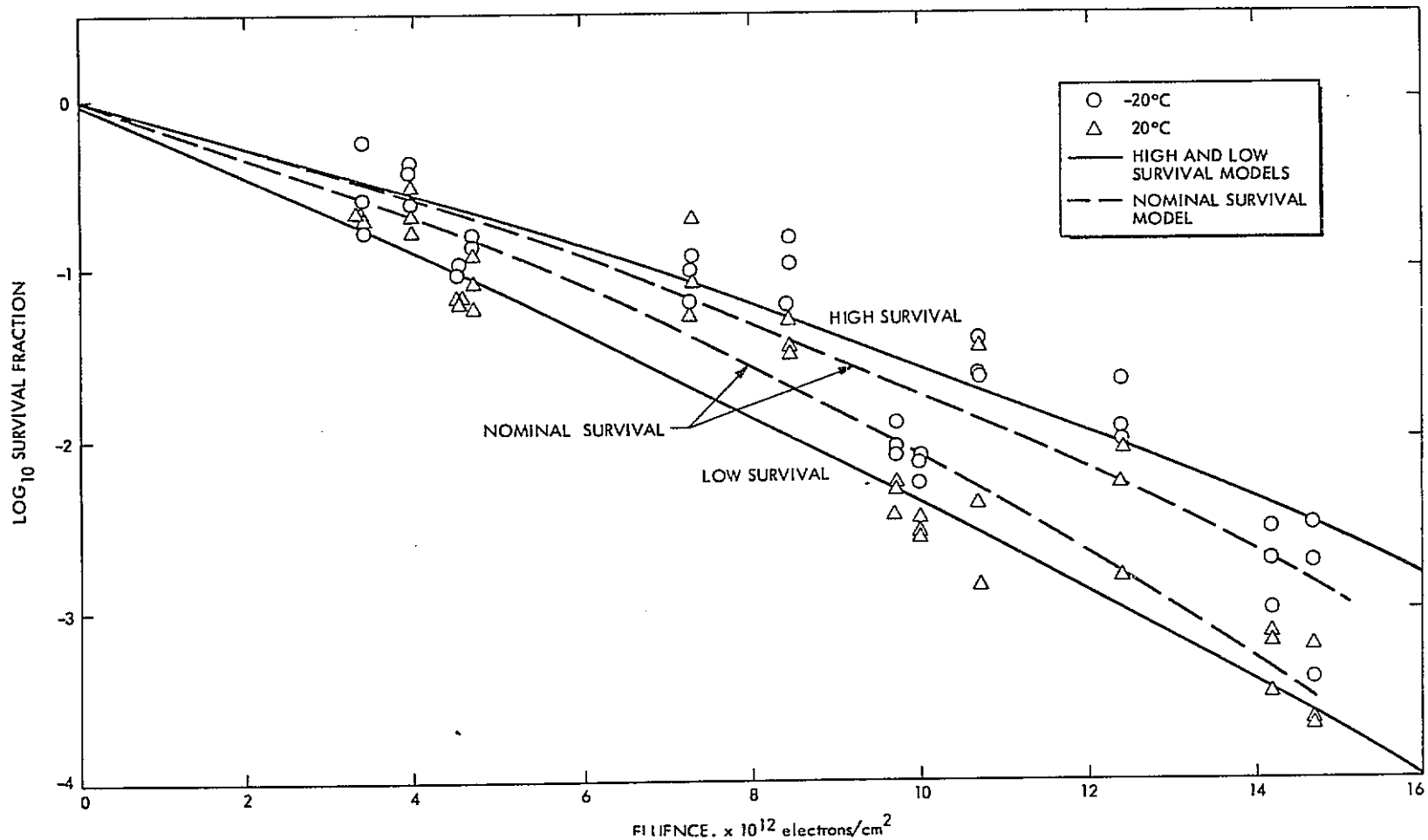

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Figure B-5 Engineering Models for Bacillus Subtilis Vàr. Niger Survival in High Energy Electron Environments